FOOD INTAKE OF SELENIUM AND SULPHUR AMINO ACIDS IN TUBERCULOSIS PATIENTS AND HEALTHY ADULTS IN MALAWI

Frode Eick

Supervisor:
Professor Gunnar A. Bjune

Co-supervisors:
Professor Asim K. Duttaroy
Dr. Kenneth Maleta

University of Oslo
Faculty of Medicine
Institute of General Practice and Community Medicine
Section for International Health
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Abstract

Title

Food intake of selenium and sulphur amino acids in tuberculosis patients and healthy adults in Malawi

Background

Tuberculosis is a world wide pandemic and a major problem for people in low income countries. The intracellular infection has a bidirectional link with under nutrition, and wasting is a common symptom of the disease. Wasting in tuberculosis patients is associated with the severity of lung disease, low serum level of selenium and higher mortality. Low serum levels may be due to low intake or high body consumption of the components. No assessment of intake of selenium and sulphur amino acids has been done in tuberculosis patients.

Objective

The main objective is to calculate and compare the intake of selenium and sulphur amino acids in tuberculosis patients and appropriately matched healthy adults.

Methodology

Participants did a one day interactive 24 hour recall at their home together with a structured questionnaire about socio economic status. Results were compared on group level. Local food was collected and analysed with an inductively coupled plasma mass spectroscopy for selenium content. 7 local samples were collected and analysed for sulphur amino acids and compared to Kenyan and South African food composition tables. The intake was calculated on this basis.

Result

There was no significant difference in intake of selenium and sulphur amino acids by the two groups. Both TB patients and healthy controls had a low intake of selenium and an adequate intake of sulphur amino acids. Controls ate more staple food but had a lower diversity in the diet than the cases. TB patients had a significant lower body mass index than the healthy controls.

Conclusion

This study indicates that the deficiencies of selenium and thiols in serum found in earlier studies may not entirely be due to a low intake of selenium and sulphur amino acids.

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List of abbreviations

µg	Microgram
AAA	Amino acid analyser
BMI	Body mass index
CAN	Calcium Ammonium Nitrogen
CI	Confidence interval
FF3	Food Finder 3
g	gram
GSH	Glutathione
GSHPx	Glutathione peroxidase
HIV	Human immunodeficiency virus
ICP-MS	Inductively coupled plasma mass spectroscopy
IR	Interquartile range
KFB	Kenyan Food Base
Kg	Kilogram
mg	Milligram
OR	Odds ratio
RDA	Recommended daily allowance
RNI	Recommended nutrient intake
ROS	Reactive oxygen species
SD	Standard deviation
SPSS	Statistical Program for Social Science
TA	Traditional Authorities
TB	Tuberculosis

1 Introduction

1.1 Country profile

1.1.1 Geography

The Republic of Malawi is a landlocked country in South-East Africa. It lies south of the equator within 9-17 degrees, and 33-36 degrees East of the Prime Meridian. It borders United Republic of Tanzania in North East, Republic of Zambia in North-West and the People's Republic of Mozambique in South. Lake Malawi stretches 475 kilometres along the north-eastern border. Other lakes are Lake Malombe and Lake Chilwa in south. In west and south there are fertile plains and mountains that peak from 1700 to 3000 metres above the sea level. The climate is sub tropical. As the country is situated below equator the weather becomes hot from September. A rainy season starts in November until April and a dry season carries on between May to October.(1)

The country is divided into Northern, Central and Southern Region and 28 districts. Six of them are in the Northern, 9 in the Central and 13 in the Southern Region. Lilongwe is the capital of Malawi and it is situated in the Central Region.(1)

1.1.2 History

The area around Lake Malawi was probably inhabited around 50-60000 B.C. Between the 1st and 4th century AD Bantu-speaking people inhabited the area. Several kingdoms were established in the pre colonial period like Maravi, Ngonde and Chikulamayembe. Portuguese people reached the area during the 16th century. A large slave trade by Arabs and Europeans took place during the 18th and 19th century. Islam and Christianity were also introduced during this period (2). In the resent past the area was occupied by United Kingdom and the British protectorate Nyasaland was established in 1891. The 6th of July 1964 people got their independence and formed the Republic of Malawi. Their first president Hastings Kamuzu Banda held the power from independence until 1994 when multiparty elections were held (3).

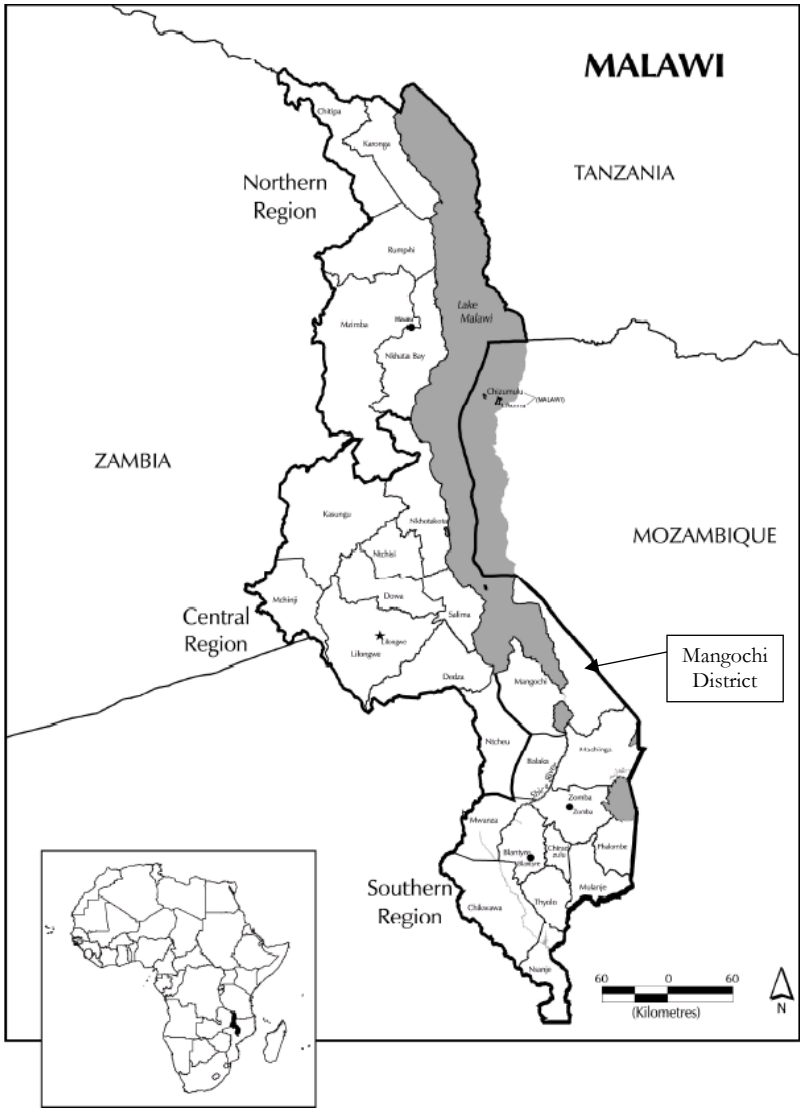

Figure 1.1: Map of Malawi

The Great Rift Valley which goes from Israel and Horn of Africa runs through Kenya, Tanzania and the entire length of lake Malawi. Mangochi district is situated in Shire Valley which is a part of Great Rift Valley. The area in Great Rift Valley is associated with volcanic and igneous rocks.

Volcanic and igneous rocks have generally a lower concentration of selenium than sedimentary rocks and the content in soil is mostly correlating with content in the rocks.(4)

1.1.3 Population and demographical characteristics

Of the 13,6 million living in Malawi there are several tribes which include Chewa, Nyanja, Tumbuka, Yao, Lomwe, Sena, Tonga, Ngoni, Ngonde, Asian and European. English is spoken in urban areas but the main languages are the official Chichewa (57%), Chinyanja (13%), Chiyao (10%), Chitumbuka (10%) and several other small languages (3). 88% of the Malawian population is living in rural areas (5).

A high proportion of the population in Malawi is young. The country has a population pyramid of a low income country with wider parameters in the young ages than the older. 17,2% of the population are aged 0-4, and 2,8% are aged 45-49. Concerning the sex distribution in Malawi, 49% of the population is male. The mean household size is 4,5 and

77% of the household heads are male. Malawi has many children, 12% or about 700 000 aged 15 or less who have lost one or both parents (1).
The population of Malawi grew from 8,0 million in 1987 to 11,2 million in 2002 which represents an intercensal growth rate between 2,0-3,3 % per year. The population density grew from 85 persons per square kilometre in 1987 to 119 persons per square kilometre in 2002 (1). Such an increase will have an impact on the economy and the ability of self subsistence of food in the country.

There is a clear difference of educational level between urban and rural areas. Only 8% of the urban women aged 15 to 49 years have no education compared to 27% of the rural women. For men 5% and 13%, respectively, have no education. In Mangochi district the situation is even worse, 43% of the women and 20% of the men have no education. These numbers reflects also on literacy in the population, 16% of the urban women (15-49y) are illiterate and 42% of the rural women. There are though much more illiteracy among older women. Among men 7% of the urban are illiterate and 24% of the rural.(1)

1.1.4 Economy and socio-economic status

Malawi is one of the least developed countries in the world and it was therefore approved in 2006 for relief under Highly Indebted Poor Countries programme. The national economy is heavily depended on funds from international institutions like International Monetary Fund and World Bank and donor nations (3). Malawi has a predominantly agricultural economy. Tobacco, tea and sugar are the main exports and in 2004 agricultural products accounted for 70% of the export (1). There are many more small holders than real estates. In comparison, 22% of the population above 15 years age is employed in the agriculture sector. Still 82% of the rural population over 15 years of age reported that farming were their main activity last 7 days. The unemployment rate in Malawi is overall 7,8% and in Mangochi 8,5% (5).

The national poverty line is set to a consumption of 16 165MK per person per year, which means about 0,3 US$ per person per day. Based on this line 52,4 % of the Malawian population is poor. The Southern Region has the highest poverty rate (60%). The poverty rate in Malawi is shown to have a high correlation with the size of the household. Poor households have larger mean household size than non-poor. The education of the household head is also correlated with poverty status. It is more likely to live in poverty if the household head have no formal education.(5)

1.1.5 Health issues and administration

1.1.5.1 Health services

The health services are provided by different actors in Malawi. The Ministry of Health is the main provider and the Christian Health Association of Malawi (CHAM) is the second biggest provider. Essential clinical services provided by Ministry of Health are free, but there may be a fee for "non-essential" services like biopsies etc. The patients have to pay for the services provided by CHAM, but the Ministry of Health is paying CHAM for certain maternal health services to keep them free of charge. In addition, there are several other NGOs working with health and health related issues. The health services are delivered by several different structures. The 66 dispensaries are staffed with a nurse or medical assistant. There are 416 health centres with varying number of trained staff and equipment and 22 district hospitals (6). The health services in Malawi are experiencing shortage of qualified staff.

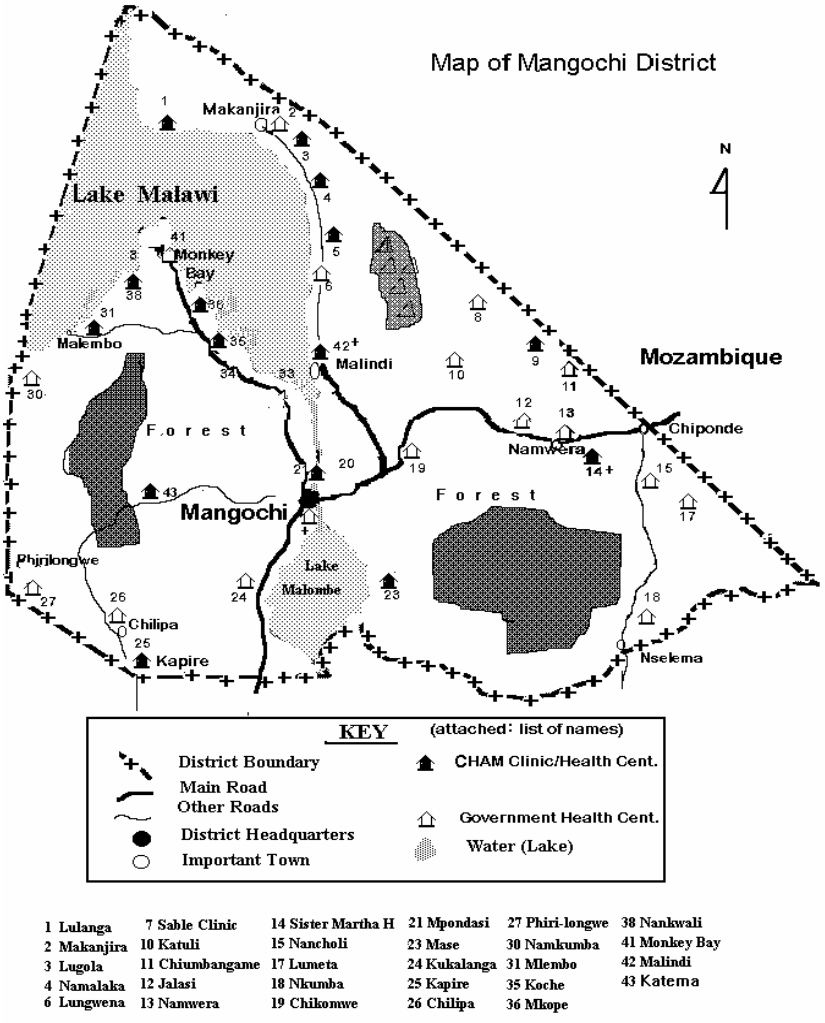

Figure 1.2: Map of Mangochi district

There is also serious shortage of essential medicines, basic medical diagnostic equipment and surgical supplies. Approximately, 54% of the rural population and 84% of the urban population has access to health services and health indicators are generally bad (6). A study assessing socio economic support for good health in Lungwena area in Mangochi district found that 48% of pregnant women had access to health facilities (distance less than 5 km) and that on average 2 socio economic determinants of health like adequate water supply or sanitation, adequate access to modern health care, literate adults, and food security were missing in each household (7).

1.1.5.2 Brain drain

Malawi is experiencing a serious shortage of health workers and there are up to 50% vacancies on established post in some health facilities. A major challenge is to attract and retain the health workers trained (6). Most of the doctors are working in central hospitals or have administrative positions. The set up of medical education is similar to British and many migrate to work or study overseas. Clinical officers with a 4 year medical education are many places doing the clinical consultations, minor surgery and patient diagnosis. Many of them are skilled but lack follow up and equipment. In Mangochi with a population of 600 000 there were about 5 doctors which gives a doctors rate of 1 per 120 000 population (8).

1.1.5.3 Human Immunodeficiency Virus

As in the rest of the countries in Sub Saharan Africa, Human Immunodeficiency Virus (HIV) has affected the population of Malawi hard. The HIV prevalence of Malawian adults between 15 and 49 years is 12 %, and it is generally higher in women than men and in urban residents than rural residents. The prevalence increases with the household's economic status, and for men also with education. Mangochi district is one of 4 districts in Malawi where the prevalence is higher than 20%, respectively 21%.(1)

1.1.6 Agriculture and the Nutrition status

The main staple food in Malawians diet is maize. Of agricultural households in Malawi 97% grow maize. 44% grow other food crops. Of those 50% grow pulses, 37% grow ground nuts, 23% grew cassava and some grow rice, cotton or other grains. Of agricultural households who raise animals, 89% have chickens and 35% have goats (5). Farming is mainly done by hand power. In Southern Region the farm size is 0,17 hectare on average (9). A small farm is more vulnerable to food insecurity. An adequate size for growing enough maize is 0,2 hectare per

household person (7). Food and Agriculture Organisation estimated that Malawi were self sufficient with maize but that 400 000 people needed assistance in 2003 due weather conditions leading to crop failure (9). Due to erratic rain falls and high cost of fertilizers Malawi experienced food insecurity three years after 2001 and had to import maize from South Africa.

The nutritional status in children and women was assessed in 2004. 48% of children were stunted, 5% wasted and 22% underweight. The average Body Mass Index (BMI) in women (15-49y) in Malawi was 22. 9% of them had a lower BMI than 18,5 which is considered as a cut off value for chronic energy deficiency. In Mangochi this percentage was 12,5. Even if this may vary year by year it is an indicator of the nutritional level in Malawians. There is high deficiency rate of micronutrients in the population. Vitamin A supplementation is given to all children age 6-59 months but only 65% received it. In this age group 73% were classified as having anaemia as well. 18% of the pregnant women reported that they took iron supplementation for over 90 days as recommended. In Mangochi only 10% did it. Of women aged 15-49 years 44% had anaemia.(1)

1.1.7 Geographical location of the study population

Mangochi district is situated in the south end of Lake Malawi and also surrounds Lake Malombe. Between these two lakes runs Shire River. The central administration is in Mangochi town which lays along the river a couple of km from Lake Malawi. The district is divided into 9 different Traditional Authorities (TA). Many from the Yao tribe live in Mangochi district and Chiyao and Chichewa are the main languages spoken. The study from Lungwena indicates that most of people are Muslims in the district contrary to general Malawi that are Christians (7). As seen in above text health and education indices are often worse in Mangochi district than the average of Malawi. There are 2 hospitals in the district, Mangochi district Hospital run by the government and St.Martin Hospital by CHAM.

1.2 Background

1.2.1 Tuberculosis and under nutrition

Tuberculosis (TB) is caused by the bacteria *Mycobacterium tuberculosis*, and is a worldwide pandemic. One third of the world's population is infected, and about 10% of these have or will develop the disease. About 5 thousand people die every day due to TB, 98% of these from the developing world (10). After the spread of the HIV pandemic, TB cases have increased even more. The prevalence of HIV in TB patients has shown to be around 70% in Malawi (11). The prevalence of TB in Malawi is 551 per 100 000 population and the mortality is 107 per 100 000 population per year, a very high mortality for a curable disease (12).

Under-nutrition has a bi-directional link with TB which has been known for a long time. The disease results in secondary wasting, and under nutrition is frequently causing immune deficiency with higher risk of getting TB. Still data on the association between TB and nutrition is scarce and none nutritional interventions were done from 1970-2000 (13). Earlier studies have documented wasting in over half of the TB patients in Malawi (14-16). Compared to healthy controls TB patients' Body Mass Index (BMI) has been shown to be significantly lower (17). Wasting in TB patients has also shown to be associated with the severity of lung disease, mortality and selenium deficiency in 3 separate studies (14-16). Out of these findings, there may be a potential for decreasing mortality and severity by improving TB patients' nutrition status through supplements or eating food with some specific nutrients which are beneficial. This theory is supported by the fact that the TB patients are deficient in micronutrients. In 3 studies from Malawi and Ethiopia, TB patients were reported to be deficient in or had significantly lower level of micronutrients than controls (14;18;19). One single study done recently has shown that supplementation of multivitamin and minerals significantly increased weight in sputum positive TB patients and reduced mortality in HIV co-infected patients (20).

1.2.2 Immune system and the role of selenium and sulphur amino acids

In intracellular infections like TB, it is primarily the cell-mediated arm of the immune system that imparts resistance and recovery. Macrophages which are phagocytic, present antigens to CD4 cells and produce cytokines, play a key role. Neutrophils are also important as

phagocytes and with their defensins and degradative enzymes. They are both important in the inflammatory response (21). The specific mechanisms in the body to eliminate TB are many and complex and largely dependant on release of various oxidative molecules. Macrophages and neutrophils release free radicals to destroy microbes. The host cell needs a strong antioxidant system to prevent being damaged by itself (22;23).

Insufficient intake of micronutrients such as antioxidants may affect the capability of the immune defence system. Selenium deficiency affects the metabolism of glutathione (GSH), and the activity of glutathione peroxidase (GSHPx), both parts of the antioxidant defence system (24;25). Selenium may also itself act as an antioxidant, through selenoprotein P and W, and affects the proliferation of lymphocytes. It is also associated with neutrophils' capability to kill pathogens (26). This capability seems reduced in TB patients (27). The two sulphur amino acids are methionine which is essential and cysteine which is semi essential, synthesized from methionine. Cysteine is incorporated in the enzyme GSHPx, is one of the amino acids in GSH and may be converted into taurine, the predominately nitrogenous compound in immune cells. The sulphur amino acids are also involved in cell replication and lipid and energy metabolism important for the immune response (23). Mycobacteria has *in vitro* shown to be sensitive to GSH (28).

1.2.3 Diet and intake

The sulphur amino acids and selenium are mainly coming from what people eat. Selenium cannot be derived from the biosynthesis of plants and the level in food items varies a lot geographically. This variation has an especially strong influence where people depend on locally grown food (29). At low sulphur amino acids intake, protein synthesis will have priority over GSH and taurine synthesis which will be reduced and the antioxidant defence will become weakened (23).

Generally sea food and organ meat like liver and kidneys are good sources of selenium. Muscle meat, Brazil nuts and cereal may be other sources (4). Sulphur amino acids are more abundant in animal and cereal proteins than legume proteins. Animal protein has also a higher net protein utilization factor and the methionine to cysteine ratio is higher than in plant sources (30). The maize protein zein contains less of certain amino acids than albumin in eggs and casein in milk. It is therefore important with a diverse diet complementing the protein

quality. In developing countries fat intake often contribute little to total energy and up to 80% of the energy comes from carbohydrates in cereal and starchy food (31).

A study from Burundi found fish as a main source of selenium and the rural population who ate little fish had lowest and a deficient intake (32). The National Food Consumption Survey in South Africa showed that children 1-9 years of age had micronutrient intake below 2/3 of the Recommended Daily Allowance(RDA) (33). A study from Mangochi district in Malawi described that the main food eaten were maize porridge ideally served with fish, legumes or green leaf sauce. It reported that maize was grown domestically and small cakes, roots, legumes, vegetables and fish were bought locally. Commercial food was consumed rarely. The Malawian rainy season lasted from November to March and people harvested mainly in April to May (34). The content of selenium in food grown in Zomba district in Malawi was similar with areas with a low concentration in soil (35). This could indicate a low intake in the population. Another study from Zomba district found that 43% of children aged 4-6 years had a deficient selenium intake. The main food groups contributing to selenium intake were cereal, fish, legumes and ground nuts, and fruit and vegetables (36).

TB is associated with poverty (37). It is likely that poor people who only can afford staple food and vegetables probably suffer from lower intake of selenium and sulphur amino acids. Earlier studies have shown TB patients being under nourished and deficient in micronutrients. The intake of selenium and sulphur amino acids in TB patients has not been investigated in detail.

1.2.4 Objectives for the study

Main objective

To calculate and compare the median consumption of selenium and sulphur amino acids per day in sputum positive TB patients and in appropriately matched healthy controls.

Part objectives:

1. To measure the content of selenium and sulphur amino acids in food items eaten by TB patients and healthy controls in Malawi.
2. To describe some of the risk factors for low selenium and sulphur amino acids intake per day in TB patients and healthy controls in Malawi.

1.2.5 Research question

Do sputum positive TB patients in Malawi have a lower intake of selenium and sulphur amino acids than healthy controls?

1.2.6 Hypothesis

H0: TB patients in Malawi do not have a significant lower intake of selenium and sulphur amino acids than the healthy population.

H1: TB patients in Malawi have a significant lower intake of selenium and sulphur amino acids than the healthy population.

2 Methods

2.1 Study area

The study area was the Mangochi district south east in Malawi. This area was chosen mainly because of the existing collaboration between the University of Oslo in Norway and College of Medicine in Malawi and also its location within Great Rift Valley.

2.2 Design

A matched case - control study design with adult sputum positive tuberculosis patients as cases and adult people as controls was used. Tuberculosis is a rare disease with a prevalence of 0,5% and the differences between the groups are the focus. Based on the objectives, the aim of the study is to give a true descriptive picture of the specific situation. To gather information, a one day interactive 24 hour recall was conducted with all the participants. The method used was from the manual "An Interactive 24-hour Recall for Assessing the Adequacy of Iron and Zinc Intakes in Developing Countries" (38). To gather additional information on the participants' socioeconomic status and farming methods, a structured questionnaire was used. Smear positivity and start of TB treatment were noted from the TB register book.

This study was done together with another study led by Heidi Arntsen who collected blood samples from these TB patients and controls. Heidi Arntsen participated also in preparing and doing the 24 hour recalls. Identified TB patients and controls were asked to join both studies but could pick one. Information on duration of symptoms is gathered from a structured questionnaire from that study and is used by permission from Heidi Arntsen.

2.3 Study population

42 TB sputum positive patients were recruited from Mangochi district Hospital in Mangochi town and St.Martin Hospital in Malindi. Both hospitals are situated within Mangochi district. Out of 102 TB smear positive patients identified, 33 could not be enrolled for the study and 2 died at arrival. Of the 67 patients, 42 were wiling to participate, 11 rejected and 14 said they were willing but didn't follow through (2 of them died). Of the controls asked, 12 did not participate even if they agreed to participate. Since it was an individually matched case control study, the 2 TB patients who did not get a matched control were excluded. The final

number was 40 matched pairs, equal to 80 participants where 3/40 patients were recruited from St. Martin Hospital. HIV testing was not done due to the consideration of TB patients as one target group for a possible nutrition intervention in future.

Inclusion criteria for the patients:

- A TB smear positive test after one Ziel-Nielsen staining.
- The participants had to be in the age group from 15 – 60 years.
- The TB smear positive patients in the intensive phase will be included in the study after 2 weeks on TB treatment.
- Living within Mangochi district

Exclusion criteria:

- Patients with other forms of TB, on retreatment and relapses were excluded.
- Being on treatment for over 2 months excluded the patient.

Inclusion criteria for controls:

- The participants had to be in the age group from 15 – 60 years.
- The controls were matched with the TB patients on age ($\pm$ 10y), sex and resident of the same village.
- Living within Mangochi district

At first the age limit was set to $\pm$ 3 years. As the diet seemed very similar in type and amount for the different age groups, and there were difficulties in finding matched controls in small villages, the limit was changed to $\pm$ 10 years, within the inclusion criteria. Many of the participants did not know how old they were and an estimate had to be done based on historical events they remembered.

2.4 Sample size

The estimated sample size was 120 participants, 60 in each group from a calculation model from Hein Stigum. This was done on the basis that selenium intake was the main exposure of TB and we wanted to study the association between low intake of selenium or sulphur amino acids and TB in terms of an odds ratio. In a study with 1 control per case, where 70% of the cases were exposed to low dietary selenium/sulphur amino acids, and 43,75% of the controls were exposed, and 60 cases and 60 controls was the estimated number of participants possible

to find, there had to be an odds ratio of 3 to make the study justifiable with a confidence level of 95%, a power of 80% and a 95% confidence interval that did not include 1. This seemed reasonable.

During the field work it came obvious that this number could not be reached due to unexpected obstacles. The ethical approval was received 28 August 2006, one month after scheduled. The Ramadan lasted from 23 September until 23 October 2006. In this period only a few 24 hour recalls were done because it was thought that the diet could be influenced by personal or relatives' fasting. The interviews were done in this period with Christian population only. The nature of the study meant that the researchers had to go home twice to the homes of the participants in rural countryside. The roads were generally in bad conditions and a short distance could take hours to reach. Very few had means to communicate like phone or radio if they had to cancel the meeting. As mentioned before 26 consented to participate but did not follow through of different reasons. Four of these participants were not found even if they had indicated which village they lived in. This meant a lot of unnecessary travelling for the researchers to reach these volunteers due to lack of appropriate logistics.

2.5 Sampling method

TB patients within the inclusion criteria were mainly approached in the hospital ward as many were inpatients for the first 2 weeks. Some that had been transferred to a health centre were tracked down when they came to receive their medication. The patients were approached at their beds by the research teams without the staff involvement.
The controls were approached in their own household after identifying what village the TB patients lived in. A random walk method was used to find the controls. The researcher found the centre of the village together with the village chief and walked randomly from the centre after spinning a bottle on the ground indicating the direction. The walk carried on to the edge of the actual village, numbering the households which were passed. A number was randomly picked from small paper notes. If a control was not detected or wanted to participate in the first household, the next rising household was chosen. If a participant was not found in the line, the researcher went back to the centre and spun the bottle again.

2.6 Ethical clearance

The study was approved by a Norwegian ethical committee at University of Oslo and College of Medicine Research Ethical Committee in Malawi after minor comments.

2.7 Data collection

2.7.1 Preparation

The researchers were introduced to the staff at the TB ward and the District Health Officer at Mangochi district Hospital. After approval from District Health Officer and the director of St.Martin Hospital to conduct the study, the District Commissioner, local police and all the village chiefs were informed about the study. One village chief refused on behalf of his villagers to participate after consulting them.

2.7.2 Research assistants

The data collection was performed by two groups, 2 men or 2 women and with 1 master student researcher and 1 local research assistant in each group. The research assistants were recruited from College of Medicine, Division of Community Medicine located in Mangochi district Hospital. The male research assistant spoke English and the 2 local languages fluently and had the same socio-economic background as the villagers. The 2 female research assistants who shared the task in the second group were nurses. They spoke English and the most urban language and had a higher socioeconomic status than most villagers.

2.7.3 Ethics

After introducing the researchers, the information form was read aloud to them in local language. This included information on the project, its purpose, possible risks, right to terminate and others. The subjects had the possibility to ask questions and time to consider it if they wanted. If willing to participate, the participants signed or thumb printed an informed consent one after the necessary information was given in local language. For the patients between 15 to 18 years, one of the parents or closest caretaker had also to sign or thumbprint the consent form if the child agreed to participate. A refusal from the child was respected. The participants were free to refuse to participate and may withdraw from the study at any time. The information given from the participants, were held strictly confidential, and only the researchers and the assistant had knowledge about this. The answers were identified by a number, and a separate paper related a specific number to the participant's name. The 24 hour record forms were stored securely. An incentive is given to the participants in the form of soap and a bag of beans to compensate for lost working hours.

After 2 weeks on treatment the participants with TB were visited at home. In identifying the controls, it was important that the identification of TB patients not should become public. There might have been stigmatisation in the society and the researcher had an obligation to protect the identities to the participants. Therefore a random walk method was chosen in detecting the controls. Additionally, in the participant information provided at the community level, it was not specifically indicated that the study was about the intake of micronutrients in TB patients but rather about the intake of micronutrients in the general population. This was done to protect the status of the TB patients as in the small communities in Mangochi. Even using the random walk method, it would have been possible for participants to know who else in their village that had been participating in the study and therefore indirectly know the TB status of the other participant.

2.7.4 Pre testing

The interactive 24 hour recall and the structured questionnaire were tested on 5 male and 5 female patient guardians at Mangochi district Hospital. The guardians were from the same socioeconomic background as the participants and spoke the same language. The procedure was followed as intended in the study. The pre test resulted in that questions about assets and the picture chart were changed. Questions about ownership of car and motorbike were removed, the question about shoes was added and some pictures were changed. This seemed more appropriate concerning the socioeconomic level in the society and would hopefully give more balanced answers.

2.7.5 Diet interview

The 24 hour recall was done in the home of the patients and controls. In the first meeting, the participants got their own plate and cup and a picture chart of usually eaten food and a pencil, together with verbal information on what to do. They were also shown the weight scale and stiff maize porridge and estimated how much they had eaten of stiff maize porridge, as an exercise. Two days after, the researcher visited them again to do the interview which lasted for about an hour. The participants were asked to tell what they ate yesterday, where they ate it and at what time. 5 salted replica models of the 3 most common relishes, rice and stiff maize porridge were used to measure the amount of food eaten. Fresh models of Pawpaw, Mango, Sweet potato, Cassava, Sugar, Salt and Ground nuts were also brought. In the

interview the participant listed what he/she ate, gave a detailed description of each item and quantified the amount with weighing the food on a kitchen scale. For drinks the participant indicated with water in the given cup how much he/she had drunken. A large cup was calibrated with levels of 100 ml and a small with levels of 50 ml. 3 calibrated spoons of different size were also brought to estimate food items like milk or spices. At the end the researcher reviewed the interview data to ensure its accuracy. At the end the questions about socioeconomic standard were read aloud to them and the research assistant crossed for what the participant answered. Because of logistical constraints 2 diet interviews of controls were done not the day after but the 2nd day after recording.

2.7.6 Anthropometric measurements

The weight measurements of the participants were done with 2 bathroom scales, and the height measurements with 2 foldable height-measure devices with 5 parts. The bathroom scale was calibrated and put on a straight wooden surface before weighing the participant. Each participant removed shoes and wore only light clothing. The participant stood straight up during height measurement after removing the shoes. The researcher checked the two bathroom scales 4 times, weighing 5 litres of water to see that they showed 5 kg. The height measure devices were also checked 4 times during the study period that they showed the same value by measuring the 2 researchers. When the height measure devises were calibrated towards a stadiometer, it showed that device A measured on average 2 cm too short. When entering the data into Statistical Package for the Social Sciences (SPSS) these 2 cm were added on the participants measured with device A.

2.8 Calculating intake

2.8.1 Recipes

For relishes commonly eaten together with stiff maize porridge, a standard conversion factor for each ingredient was calculated from the recipe of the relish. The relishes were fish, kidney beans or green vegetables together with tomato, onion and salt.

5 – 10 women were selected from the hospital guardian area to cook a relish from a standard recipe. They were told what ingredients to add and given money to buy an amount of food for consumption by 4 people. When bringing the raw food, it was weighed together with the

cooking pot. The women then cooked the food without supervision and the cooked food was then weighed again. The raw weight of each ingredient was divided with the total weight of the cooked food. After the 24 hour recalls the average of this factor was multiplied with the weight each participant reported they had eaten of a relish.

2.8.2 Conversion factors

An average was calculated out of food found in the main market in Mangochi town for the food which was not brought to each 24 hour recall. This was done for food items like tomato (89g), banana (24g), vegetable oil (44g), okra (10g), masau (4,5g), bread slice (27g), dry fish (20g), scone (60g), boiled egg (60g), onion (small 16g and big 80g) and potato (53g). Meat, large fish and occasionally paw paw and mango were measured with model clay. Patients formed how much they had eaten of the food item with the clay. It was then lowered in a measuring cylinder with water to measure how many ml's of the food item they had eaten. The ml's were then multiplied with a factor, 1,1 for meat (calculated from pig meat), 1,08 for fish (calculated from *Chambo* fish) and 1,03 for fruit (calculated from Paw paw) to estimate the weight.

For sugar cane and corn cobs a factor was calculated of how many grams eaten per cm. The food item was weighed and measured raw, then prepared and eaten by 10 local men before the inedible parts remaining were weighed. For sugar cane and fried maize the inedible parts left were subtracted from the total fried weight (maize) and raw weight (sugarcane), and the sum divided on the length of the cob/cane. The factor, g/cm which was based on the average of 10 randomly chosen cobs and sugarcanes was multiplied by the length the participants answered they had eaten. The boiled maize cob factor was based on the average of 8 cobs. The inedible parts were subtracted from the total boiled weight and the sum divided on the length of the cob. The sum was multiplied with raw weight divided by boiled weight. This factor, g/cm was multiplied by the length the participants answered they had eaten.
For items like cow milk, milk powder, soft maize porridge, rice, sweets, cake, cabbage, soybean porridge, the conversion factors in the software program Food Finder 3 (FF3) was used. 1 sachet of milk powder was measured to 22,5 gram and used as a reference. 2 values of fried rice indicated as "half" was set as the average of measured maize cobs of 8cm, multiplied with g/cm.

2.8.3 Data handling

Intake of selenium was calculated through plotting each individual 24 hour recall into an Excel sheet and summarizing the intake of selenium from each food item eaten by the individual. The same method was done for sulphur amino acid intake. Even with a low diversity concerning the main diet, a wide range of food was seen in the less eaten food items. A generalization of this diversity was done because of the lack of all values in the data and to make it easier to handle the data without losing to much information in the calculation of intake and the proportional intake form each food type. Different type of vegetables like Chinese cabbage, different leaves, black jack vegetable and cabbage were considered with the value of Chinese cabbage, the most common vegetable. Mandasi and scone were considered as bread. African cake was considered as brown maize porridge. Wild meat was considered like goat meat and 4 kinds of beans eaten by 1 participant each were considered as kidney beans. “Mahewa drink” was considered like milk, and tea, orange squash and coffee as water. For some few data that was missing, the mode was used as a reference. 2 missing values of mango were set to 155g and 1 missing value of sugar was set to 33g. The mode was also used where a food item lacked specification. Sugar was considered as brown sugar and maize porridge as white maize porridge in the computer programs SPSS and FF3.

Local raw food values were used to calculate the selenium intake except for white and brown maize porridge where cooked values were used. Values from FF3 were used for milk, chicken, goat meat and milk powder as local values for these food items eaten were not collected.

In calculating the intake of sulphur amino acids local raw values for *Usipa* fish, white and brown maize flour, gruel and kidney beans were used. For white and brown maize porridge, local cooked values were used. Raw values form other fish and food items were taken from either FF3 or Kenyan Food Base (KFB). Most of the values used were from FF3 and supplemented with values from KFB where FF3 had missing values.

2.9 Food samples

2.9.1 Sulphur amino acids

The food samples for analysis of sulphur amino acids were bought from local vendors at the main market in Mangochi town. It was assured verbally that the food samples were coming from Mangochi district. Uncertain vendors were excluded. The collected samples were then prepared and frozen down to minus 20 degrees C and kept for 2 months. Preparation included removal of inedible parts of the food sample and mixing with a mechanical blender (Braun). The equipment was washed with tap water between each sample and dried with tissue paper. The frozen food samples were then transported in a cold box to Norway for analysis.

7 food samples were chosen for analysis to compare values with the Kenyan and South African food composition table instead of analysing all the different local food items as planned. This was done as it seemed that the geographical variation was not large and limited funding. To analyse the total content of cysteine and methionine the standard EU approved method was used (39;40). The sample was oxidised with performic acid-phenol and stored at 0 degrees C for 16 hours. Then the excess oxidation reagent was neutralised by the addition of sodium disulfite. 6M Hydrochloric acid containing phenol was added and boiled for 23 hours to hydrolyse the sample. It was then washed with citrate buffer and cooled down. Sodium hydroxide solution was added carefully and pH adjusted to 2,20 before filtrating. To analyse for the sulphur amino acids a Biochrom 20 plus amino acid analyser (AAA) with ion exchange column was used. The sample (30µl) was injected with an auto injector to an ion exchange column. To separate the different amino acids citrate buffers with varying pH and ion force were pumped into the column. The temperature of the column changed from 50-90 degrees C to foster the separation. When the amino acid reached its ionized peak, it was released from the column. Quantification happened through adding Ninhydrin when reacting with the amino acids visualised products that were detected with a photometer. The absorption of light was done with wavelengths of 570nm and 440nm. The amount of coloured product visualised correlated proportionally with the amount of the amino acid released. The concentrations of the amino acids were plotted as a series of peaks. The time of retention of a peak identified the amino acid.(39;40) These analyses of cysteine and methionine were done by Analycen Inc. in Moss, Norway

2.9.2 Selenium

The food samples for selenium analyses were collected from households, farmers and local markets in Traditional Authority Mponda, in Mangochi district. TA Mponda was the TA surrounding Mangochi town. The food that was not possible to find grown locally were bought from markets in Mangochi town. The samples were then kept for one week maximum in a refrigerator at 4 degrees C before they were transported in a cold box to Bunda College of Agriculture in Lilongwe and prepared. Preparation included removal of inedible parts and weighing. Preparation equipment was washed with distilled water between each sampling. Composite samples were then dried at 70 degrees C in paper bags in an oven. The most liquid samples like banana, tomato, paw paw, gruel and mango were dried in metal cups in the oven. The samples were then stored in paper bags in dark for 1 month until brought to Norway in plastic bags. Food samples of cow meat and milk, and goat meat were lost due to logistical mistakes.

Food samples were dried for 3 days at 105 ºC in an oven, weighed, and milled to <1-mm particle size. The total selenium concentration in samples were determined at the Norwegian University of Life Sciences, Department of Plant- and Soil sciences by an ultraclave microwave digestion (Easy Clave 5, Milestone Srl., Italy) of 0.5 g milled samples at 250 ºC for 15 minutes in a mixture of 3.5ml sub-boiled ultra pure HNO_3 (approx. 69 %) (Merck KGaA, Germany) and 2.0 ml deionised H_2O. All samples were added tellurium as internal standard and diluted to 50 ml and stored dark and cold (4 ºC) prior to analysing. Samples were diluted 1:1 with 4 % ethanol solution (Vinmonopolet Inc.) and selenium determined by inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer, Sciex Elan 6000). The method was calibrated using standard solutions prepared from certified selenium standard (Spectrapure standards AS, Norway) and validated using the certified reference material Bush branches and leaves NCS DC 73348 (China National Analysis centre for Iron and Steel, China) and Dogfish muscle Dorm-2 (National research council of Canada, Canada).

2.10 Operational definitions of variables

Conceptual definition of variable	Operational definition of variable	Scale of measurement	Method of verification
Age (numerical)	Age at last birthday	Discrete	Interview
Sex (categorical)	Sex at birth	Nominal: Male/Female	Interview
Religion (categorical)	Belief	Nominal: Christian/Muslim/Other	Interview
Nutritional status (numerical)	The ratio of weight to square height (Body Mass Index)	Continuous	Measuring scale and board. To the nearest cm and 0,1 kg. Unit: kg/m²
Pulmonary TB (categorical)	Detection of MTB in sputum measured by Ziel-Nielsen staining	Nominal: Positive/Negative	Microscope in laboratory
Food diversity (numerical)	Numbers of food types eaten in a day, not water.	Discrete	Interview
Food amount eaten (numerical)	Amount of eaten food for each category last 24 hours	Continuous: Different food models weighed	24 hour recall Unit: g
Food type eaten (categorical)	Food drunk and eaten the last 24 hours	Nominal: Maize, fish, mango, cassava, cow milk, etc.	24 hour recall
Content of sulphur amino acid in food (numerical)	Level of sulphur amino acid in an food item	Continuous	Biochrom 20 plus AAA, Unit: mg/g
Content of selenium in food (numerical)	Level selenium in an food item	Continuous	ICP-MS, Unit: µg/g
Socio-economic status (categorical)	The household-ownership of a mattress, radio, bicycle, blanket, TV and shoes.	Nominal: Yes/No and then converted to ordinal Very poor: 0-2 assets Poor: 3-4 assets Fair: 5-6 assets	Interview

Conceptual definition of variable	Operational definition of variable	Scale of measurement	Method of verification
Literacy (categorical)	Know how to read	Nominal: Yes/No	Interview
Education (numerical)	Number of school years completed	Discrete and converted to ordinal: None: 0 years Primary: 1-4 y Primary: 5-8 y Secondary or higher: 8 y <	Interview
Occupation (categorical)	Present work	Ordinal: 6 categories reduced to 3: Farmer Non farmer Farmer and other	Interview
Socio-economic status (categorical)	Ownership of animals in household	Nominal: Yes/No	Interview
Socio-economic status (categorical)	Type of house flooring in household	Nominal: Sand, dung or earth / Wood or cement	Interview
Socio-economic status (categorical)	Ownership of land	Nominal: Yes/No	Interview
Socio-economic status (categorical)	Type of drinking water	Nominal: Tap or borehole/ Unprotected well or lake	Interview
Socio-economic status (categorical)	Time to water source	Ordinal: < 15 minutes 30 minutes 45 minutes > 1 hour	Interview
Socio-economic status (categorical)	Type of toilet	Ordinal: None Traditional pit latrine Trad. Pit latrine w/sanitation platform Flush toilet	Interview

2.11 Statistical analyses

Wilcoxon signed rank test was used to compare the intake of selenium, methionine and cysteine in the two groups. It is also used to compare diversity, the amount eaten of certain food items, duration of crop, iron and iodine intake. As TB patients and controls were matched conditional logistic regression were used to compare the socio economic characteristics and if there were any risk factors having TB. A paired sample T-test is used comparing means of BMI between the groups, times eating during a day and fat intake. Mantel-Haenszel Common odds ratio estimate was used for cross tabulation of illness and farming practises. Spearman Bivariate correlation was used to assess BMI to treatment duration, BMI to duration of symptoms, BMI to intake and intake to date of interview. Logistic regression was used looking for risk factors having one day deficient selenium or cysteine intake.

2.12 Software

The software used in the statistical analyses was SPSS 14.0 for Windows (SPSS Inc, Chicago, USA). South African food composition table in software programme Food Finder 3 (Medical Research Council, South Africa) and Kenyan Food Base, food composition table (World Food Dietary Assessment System, version 2.0 Office of Technology Licensing, University of California Berkeley, USA) was used as references towards each other and the local analyses, and as a base for sulphur amino acid content in food items. FF3 was used as well to calculate iron, iodine and fat intake by the participants. Microsoft Office Excel 2003 sheet (Microsoft Corporation) was used to calculate the intake of selenium, methionine and cysteine and the proportion of contribution by each food item.

3 Results

3.1 Description of sample

This study included 80 participants with 40 sputum positive patients and 40 controls. The response rate for TB patients was 63 % of the ones invited. There are no numbers for the control group but it was probably lower than in the TB group.

3.2 Demographics and socio economic characteristics

3.2.1 Demographics

Of the 80 participants there were 38 women and 42 men. The participants were equally shared between the 2 groups with 19 women and 21 men in each, as they were matched. The mean age was in the TB patients group 33,1 years (Standard deviation (SD) 9,74) and 31,6 years (SD 9,62) for the controls. The distribution of age was quite similar in the 2 groups with a slightly higher proportion of younger participants in the control group as seen in table 1. The youngest participant was 15 years and the oldest 56.

Table 3.1: Age distribution of participants

			Group		
			TB sputum positive	Control	Total
Age group	15-24	Count	8	10	18
		% within Group	20,0%	25,0%	22,5%
	25-30	Count	8	12	20
		% within Group	20,0%	30,0%	25,0%
	31-39	Count	13	10	23
		% within Group	32,5%	25,0%	28,8%
	40-60	Count	11	8	19
		% within Group	27,5%	20,0%	23,8%
Total		Count	40	40	80
		% within Group	100,0%	100,0%	100,0%

All the participants belonged to either Christianity or Islam. Of the total sample 70% were Muslims and 30% Christians. There were a number of Christian sub churches. 34 of the participants were living TA Mponda, while 22 were staying in TA Chowe. Mangochi District Hospital and St. Martin hospital were situated in these two areas. 20/80 participants were living in urban areas. 8 participants lived in TA Chimwala and 8 in TA Jalasi while 4 were

living in TA Makanjira and 4 in TA Nankumba. None of the participants lived in TA Katuli, TA Mbwananyambi or TA Namabvi.

3.2.2 Socio economy

Most of the participants had very few "luxury" assets except clothing, a mat and cooking utensils. Of the 7 items asked for on a household basis 85 % reported they had a blanket and 84 % shoes. About half of the participants had a radio, but only 9 % owned a television. 1/3 shared a mattress in their household and 1/3 owned a bicycle, the most common transportation. Only one participant owned a boat.

80 % of the TB patients and 85 % of the controls told they were doing farming. While most of TB sputum positive patients were doing subsistent farming only, many of the control farmers had another income. 90 % of the participants owned their land where they were farming. The rest reported they rented it.

Illiteracy was common in both groups and almost half of the participants could not read. 12 of the 18 illiterate TB patients (2/3) and 9 of the 16 controls (3/4) were female. In proportion this means that 12/19 (63 %) of the female and 6/21 (29 %) of the male TB patients were illiterate. In the control group 9/19 (47%) of the women and 7/21 (33%) of the men did not know how to read.

By unstructured observation most people lived in houses made of sun dried or the more expensive oven burned bricks. Many had quite small houses (ca 30 m^2) with a fenced backyard. Common roof were grass or iron sheet depending on cost but also comfort. Table 3.2 shows how many had cement floors compared to sand/dung or earth. Totally 76% had sand/dung or earth as floor in their home. None had wooden floor in their home. 95% of the participants reported that they got their drinking water from a tap or borehole. Very few if any had a water tap in their house but a closed borehole with a pump was common in almost every village. The time walking to waters source did mainly take less than 15 minutes, though 15% used half an hour and 5% 45 minutes or more. This was mainly a woman's job and many water pumps acted as meeting places for women and children. The sanitary conditions were poor. 83 % had a traditional pit latrine which is more or less a hole in the ground. 15% had a latrine with sanitation platform, a cement floor with a hole which is safer and easier to clean if anyone misses the target.

Table 3.2: Characteristics of participants

	TB sputum positive patient (N= 40)	Control(N= 40)	P-value
Participants' characteristics			
Religion			> 0,05
Christians	13	11	
Muslims	27	29	
Literacy			> 0,05
Yes	22	24	
No	18	16	
Occupation			> 0,05
Farmer	24	15	
Non farmer	6	8	
Farmer and other	10	17	
Own land			> 0,05
Yes	35	37	
No	5	3	
Household characteristics			
Assets			> 0,05
Very poor	18	18	
Poor	13	15	
Fair	9	7	
Drinking water			> 0,05
Tap or borehole	37	39	
Unprotected well/lake	3	1	
Time to water source			> 0,05
<15 min	32	32	
30 min	6	6	
45 min	1	2	
> 1 hour	1	0	
Flooring			>0,05
Sand/dung or earth	28	33	
Wood or cement	12	7	
Toilet			> 0,05
None	1	0	
Traditional Pit latrine	32	34	
Trad. Pit latrine w/san plat	6	6	
Flush toilet	1	0	

3.3 Food and diet

The most common hot meal was stiff maize porridge (*nzima*) of white flour, with a cooked relish of fish, green vegetable or beans, together with tomato, onion, salt and vegetable oil. This was commonly eaten 1 to 3 times per day. Of in between snack roasted or boiled corn cob, sugar cane and fruits like mango and banana were common. Many participants drank tea in the morning with sugar, either black or with milk. Some put the tea leaves directly in hot cow milk. Gruel, a sweet and thick non alcoholic beer of sorghum was commonly drunk during the day. Firewood was considered as expensive and many soaked the rice for a couple of hours instead of boiling it.

3.3.1 Intake

Comparing the intake of methionine, cysteine and selenium by the two groups there was no significant difference in any of the components, methionine (P-value = 0,440), cysteine (P-value = 0,343) and selenium (P-value = 0,893). This means that the null hypothesis is not rejected. The median selenium intake was 44 µg per day by the TB group and 46 µg per day by the control group. The interquartile range (IR) was 30 for the cases and 28 for the controls. The median consumption of methionine by the TB group was 1,345 g per day (IR 0,919). The median intake by the control group was 1,302 g per day (IR 1,019). For cysteine the median intake by the TB patients was 0,922 g per day (IR 0,779). The control group had a median intake of 0,922 g per day as well with an interquartile range of 0,594.
TB patients had a higher fat intake than controls with a median of 48gram (IR 51) and 38 gram (IR 30) respectively. (P-value>0,05)

3.3.2 Meals

TB sputum positive patients ate on average 4,6 times per day included snacks with a standard deviation of 1,5. The controls had a slightly lower mean with 4,2 times per day (SD 1,3). There was no significant difference between the two groups (p>0,05).

3.3.3 Diversity

The median number of how many different food types the participants ate during the recording day excluding water by TB patients were 12 (IR 6) and 10 (IR 5) for the controls. TB sputum positive patients have a significant higher diversity in the diet than the controls (P-value = 0,049).

3.3.4 Common eaten food

Table 3.3: Number of participants eating each food item.

Food item	TB patients n=40	Controls n=40	Seasonal
Stiff maize porridge	40	40	Partly
Ionized salt	40	40	
Water	39	40	
Tomato	37	37	
Vegetable oil	30	28	
Mango	26	26	Yes
Tea	27	23	
Sugar	31	25	
Onion	18	16	
Maize cob	15	13	Partly
Usipa fish	13	13	
Scone	15	11	
Gruel	10	11	
Mandasi (Fried scone)	11	10	
Banana	14	7	
Rice	13	6	Partly
Kidney beans	13	4	Partly
Ground nuts/ flour	11	6	Partly
Cassava	10	8	Partly
Chinese cabbage	6	10	
Kambusi fish	8	9	
Utaka fish	9	6	
Cow milk	10	3	
Milk powder	8	4	
Pumpkin leaves	6	5	
Okra	3	8	
Paw paw	8	3	Yes
Turnips leaves	7	4	
Sugar cane	6	4	
Irish potato	4	6	Partly
Orange soft drink	6	2	
Chambo fish	4	4	
Goat meat	3	5	
Monkey bread fruit	3	4	Yes
Mcheni fish	3	4	
Egg, chicken	3	3	
Pigeon peas	3	2	Partly
Banana/African cake	4	1	
Bread	2	2	
Sweet potato	2	2	Partly
Chicken	0	3	
Coca cola/Fanta	1	3	
Curry powder, red/ yellow	0	3	
Mahewa (milk drink)	2	1	
Coffee	2	0	
Other fish	7	2	
Other vegetables	6	3	
Other fruit	4	3	Yes
Other legumes	2	3	Partly

3.3.5 Proportion of intake

Maize porridge was eaten by all participants with a median intake of 681 gram (IR 488) in the TB group and 812 gram (IR 445) in the control group. This is a significant difference of amount (P-value = 0,006). Dividing into brown maize porridge and white maize porridge the tendency persisted in both food subtypes but not significantly (P-value > 0,05). 18/40 of the TB sputum positive patients ate brown porridge and 24/40 ate white porridge. In the control group 20/40 ate brown porridge and 23/40 ate white porridge. Brown porridge is made of brown maize flour where the corns are grained as they are after drying. The preferred and less tasty white porridge is made of soaked corn where the outer layer, bran is removed before graining. The level of sulphur amino acids and selenium was not high in stiff maize porridge, but a high intake of it made it an important contributor of the components.

Of other commonly eaten food median salt intake was 7,7gram (IR 10,4) by TB patients and 7,2 gram (IR 8,3) by controls. Controls had a slightly higher sugar intake with a median of 33gram (IR 18) compared to 30gram (IR 23) by TB patients. Unrefined sugar was more common than refined. TB patients had a median intake of water of 935ml (IR 840) compared to 900ml (IR 519) by controls. No significant difference was found with a significance level of p-value > 0,05. Tomatoes, onion and vegetable oil were eaten often as well but all of these contributed little to intake as the content of selenium and sulphur amino acids was very low.

Food items like fish, goat meat, chicken meat, cow milk powder, chicken egg and ground nuts items had especially high levels of methionine and cysteine. Fish had the highest level of selenium and especially the *Usipa/Cyprinids* type. This was one of the cheapest available fish on the market. Some legumes, ground nuts and herbs had high levels as well. All these food items were either eaten seldom or of little quantity. Still some of these food items with high concentration of sulphur amino acids and/or selenium were main contributors of the component in the diet related to amount eaten.
32/40 TB sputum positive patients ate fish with a median intake of 33 gram (IR 43) compared to 30/40 controls with a median intake of 42 gram (IR 33). 13 of these in both groups ate *Usipa* with a median intake of 20 gram (IR 15) by the TB group and 24 gram (IR 25) by the control group. Ground nuts were eaten by 11/40 TB patients compared to 6/40 controls with a median intake of 34 gram in both groups (TB IR 63)(Co IR 56). Only 3/40 controls ate chicken meat with a median intake of 55 gram (Range 59). 3/40 TB patients ate goat with a median intake of 66 gram (Range 75) compared to 5/40 controls who had a median intake of 110 gram (Range 133). 8/40 TB sputum positive patients used milk powder compared to 4/40

controls with a median intake of 23 (IR 11) and 16 grams (IR 31) respectively. 3/40 persons ate egg with a median intake of 120 gram (TB Range 75)(Co Range 90) in each group. There was no significant difference between TB and control group of amount eaten of these food items (p-value > 0,05).

Mango and cow milk with rather low content of selenium appeared to be important sources of the intake. Mango was eaten by 26/40 participants in both groups. The amount of intake differed where TB patients had a median of 296 gram (IR 365) compared to the controls who had a median intake of 401 gram.(IR 433) Cow milk was eaten by 10/40 TB patients and 3/40 controls with a median of 250 gram (Range 362) and 300 gram (Range 125). No significant difference was found with a significance level of p-value > 0,05.

A full overview over the sources of selenium, methionine and cysteine in the participants' diet is presented in Figures 3.1-3.6. In Figure 3.1 we see which food type is representing the sources of selenium intake in TB patients' diet. Fish represents 34% (*Usipa* 13%), maize 23%, mango 12% and milk 5% of the total selenium intake in TB patients. In Figure 3.2 the controls' total selenium intake is divided and the main sources are fish 33% (*Usipa* 18%) maize 26%, mango 17% and meat 4% (chicken and goat). Other fish, mainly large type represented more of the fish intake of TB patients than controls. These fish types were not commonly observed in the market as the other types of fish.
In Figure 3.3 the total methionine intake by TB patients is presented. Maize represents 34% of the intake, fish 33% (Usipa 9%), milk 7%, bread 6% and legumes/gruel 5%. The main sources in the controls' methionine intake are maize 41%, fish 29% (*Usipa* 13%), meat 9% and bread 5% as seen in Figure 3.4.
Concerning cysteine, maize 42%, fish 15% (*Usipa* 5%), bread 10%, legumes 7%, rice 6% and ground nuts 5% are the main sources in TB patients' intake (Figure 3.5). In Figure 3.6 the cysteine sources in controls' diet are presented. Maize 51%, fish 15% (*Usipa* 8%), bread 8%, meat 6% and egg 5% are the main contributors.

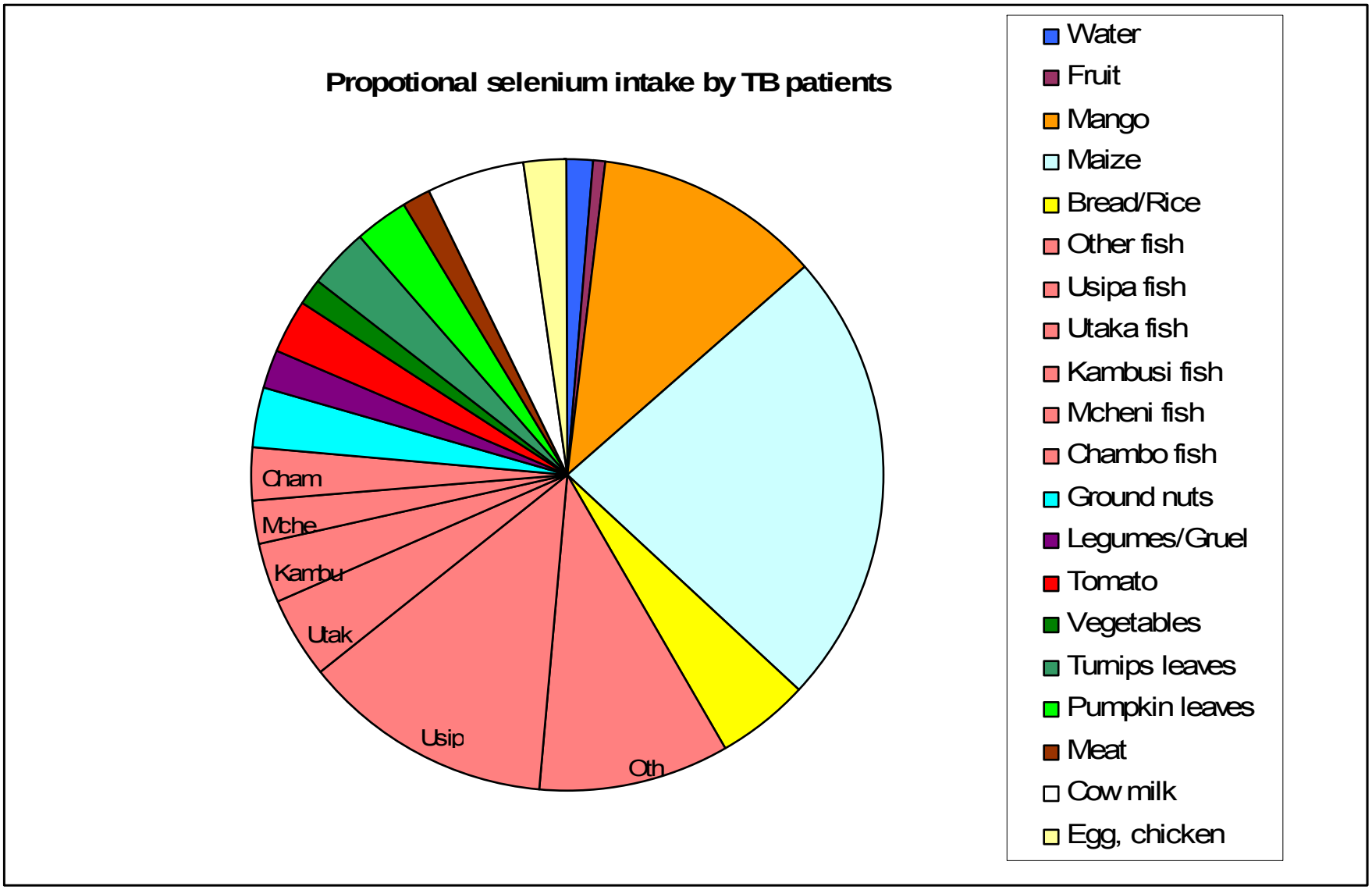

Figure 3.1: Contribution of food types in selenium intake by TB sputum positive patients.

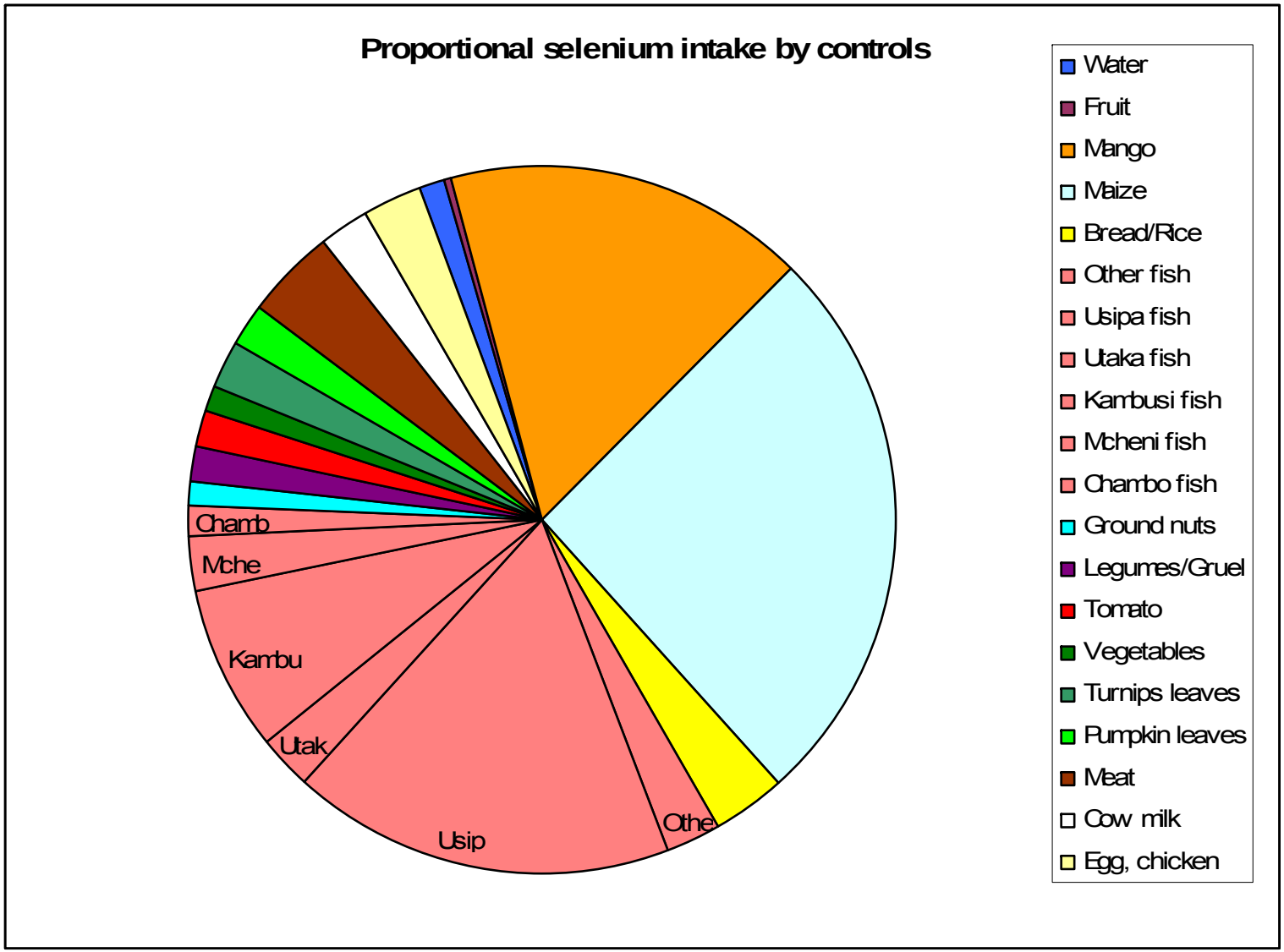

Figure 3.2: Contribution of food types in selenium intake by controls.

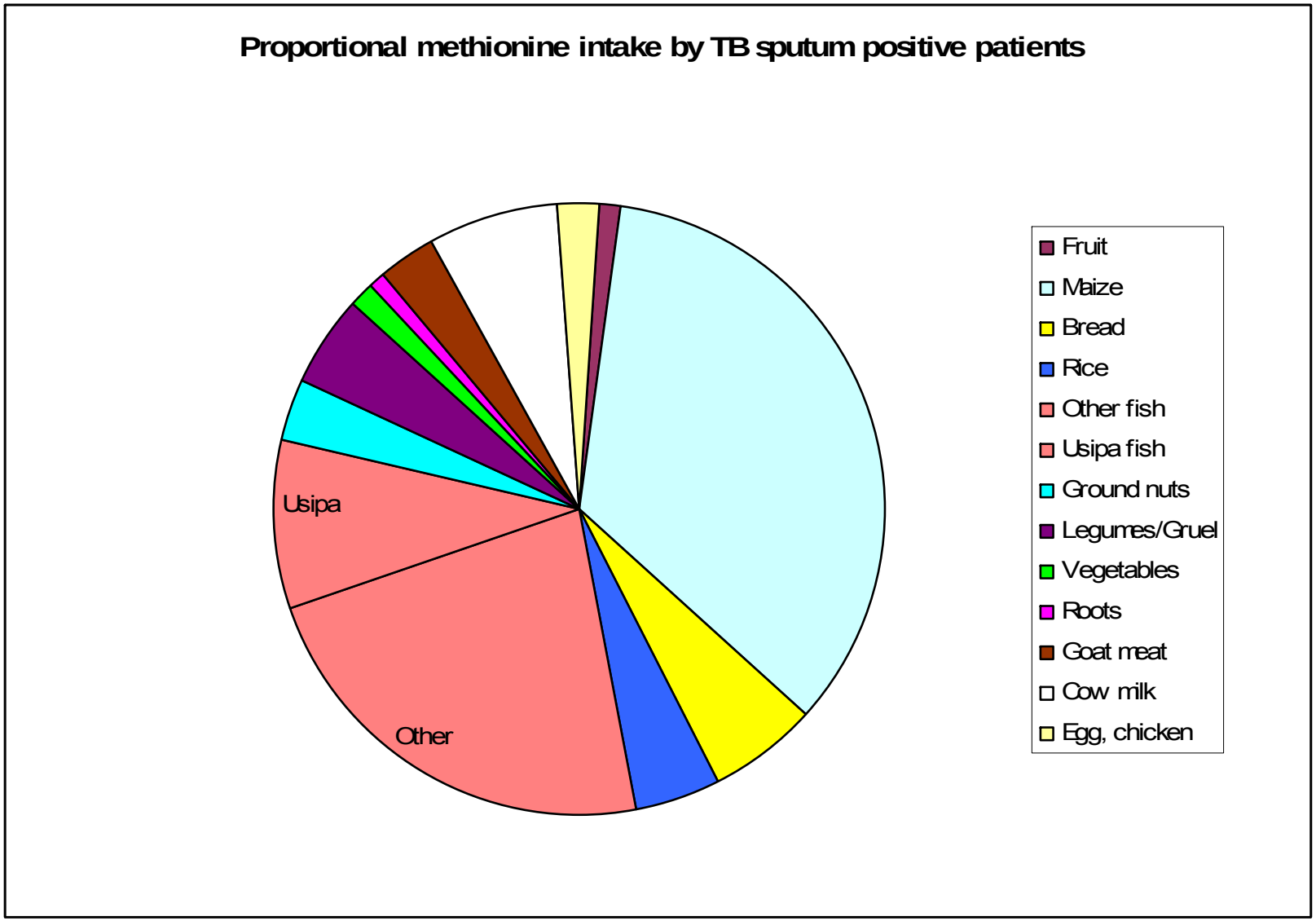

Figure 3.3: Contribution of food types in methionine intake by TB sputum positive patients.

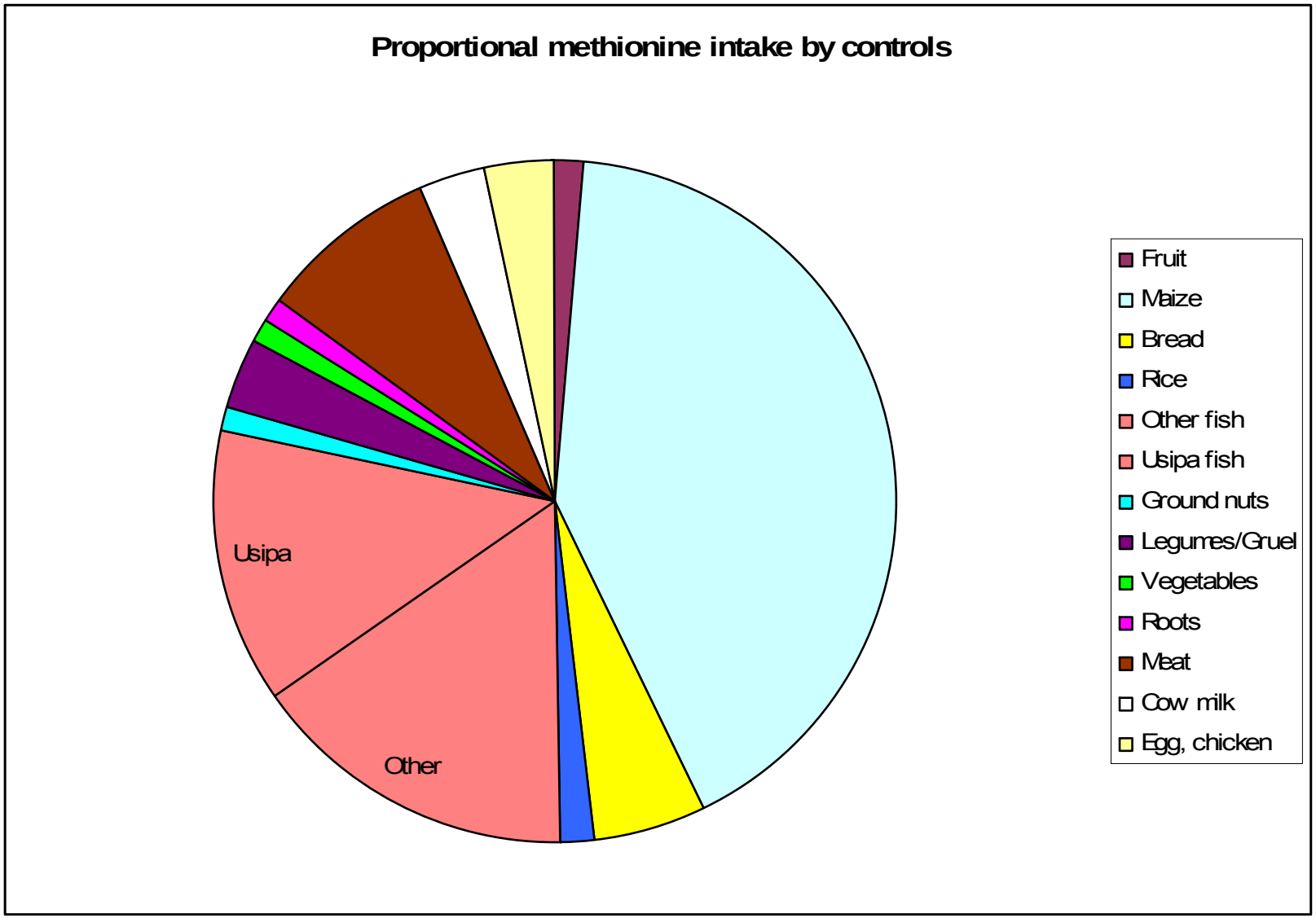

Figure 3.4: Contribution of food types in methionine intake by controls.

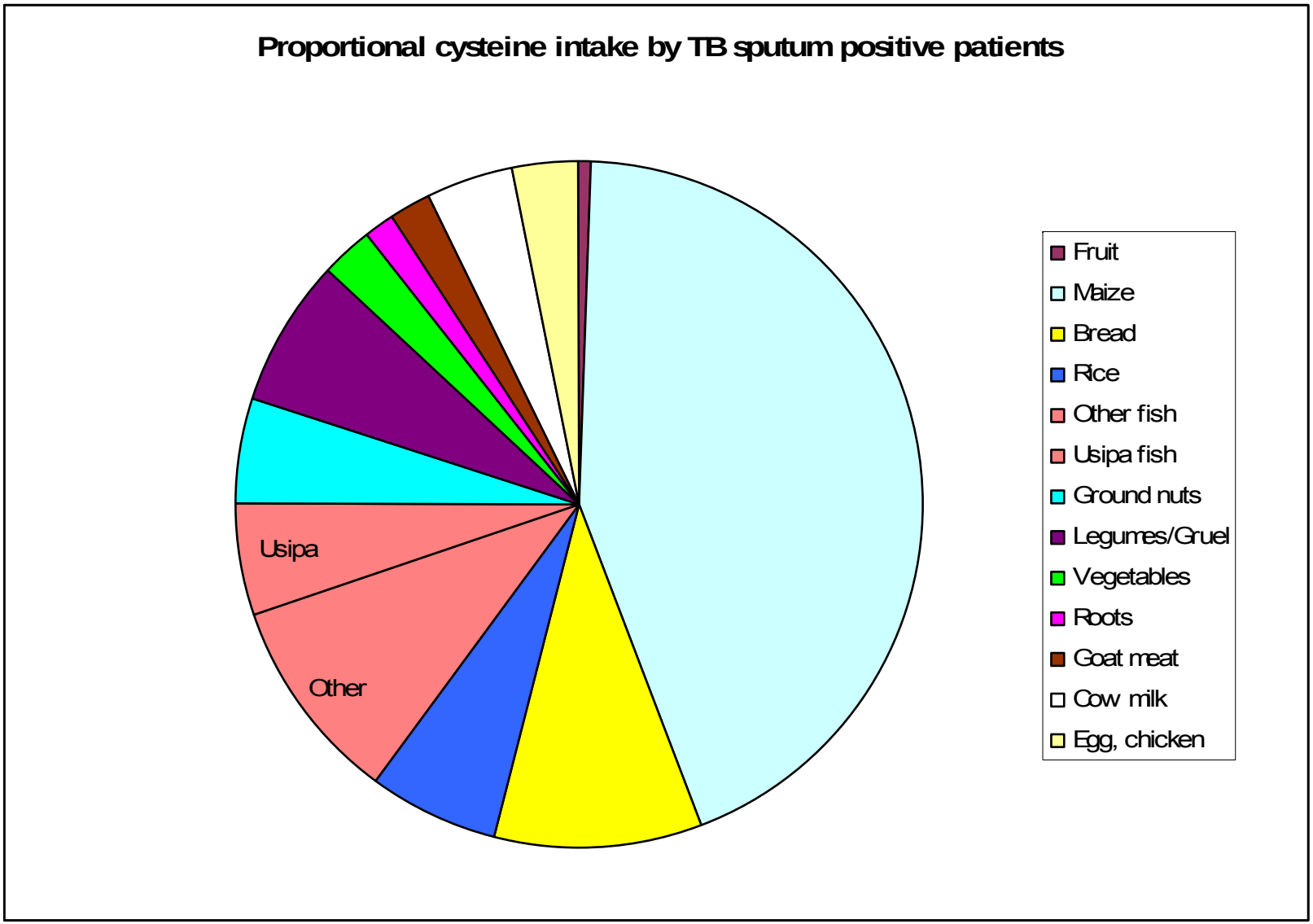

Figure 3.5: Contribution of food types in cysteine intake by TB sputum positive patients.

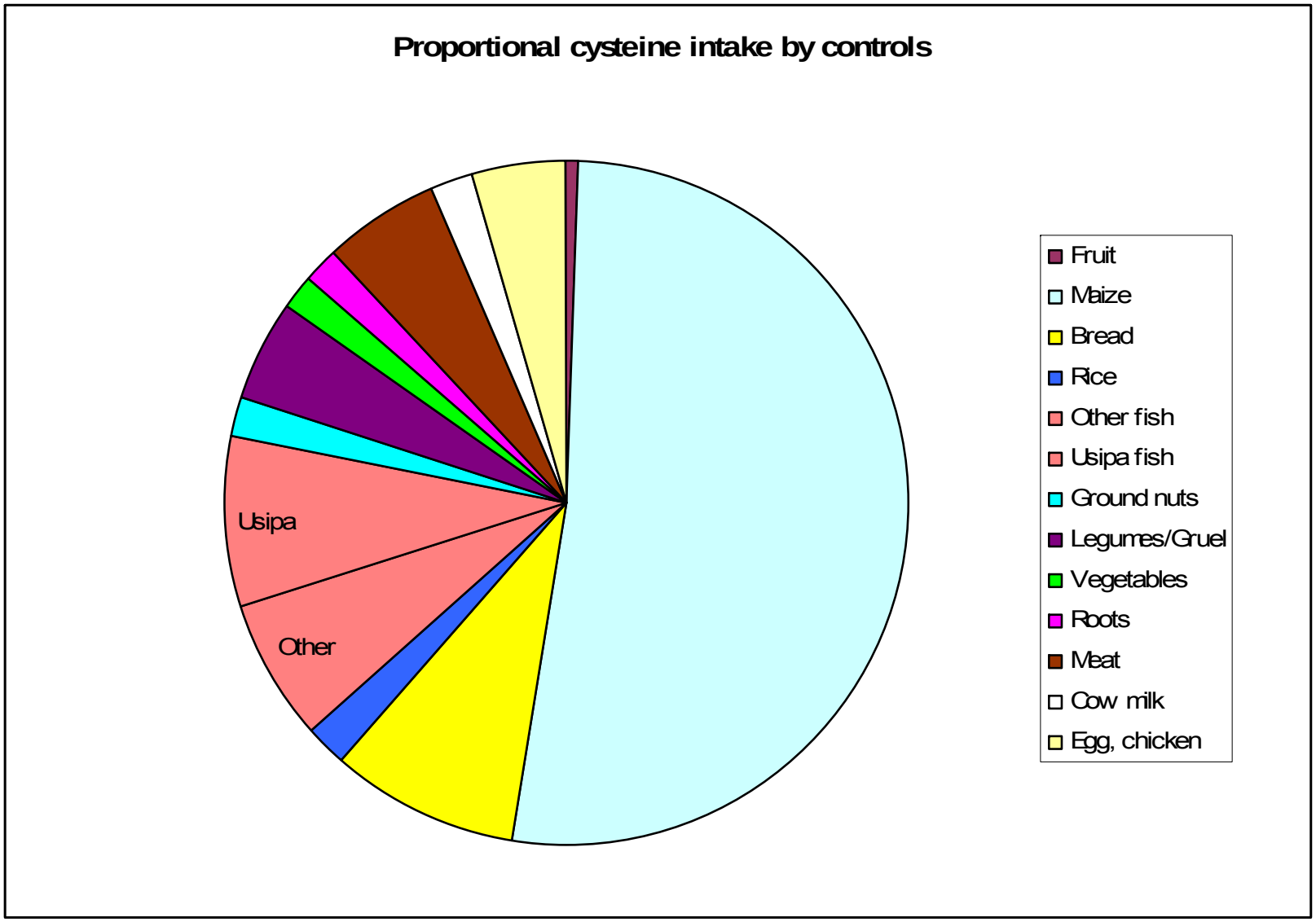

Figure 3.6: Contribution of food types in cysteine intake by controls.

3.3.6 Outliers

As seen in the Figures 3.7 and 3.8, there were some outliers concerning the amount of methionine and selenium intake. A group of 4 TB patients have close to 4 gram of methionine intake. 2 TB sputum positive patients and 1 control have over 100 µg of selenium intake. These outliers are similar to the outliers in Figure 3.9 where the amount of fish is presented.

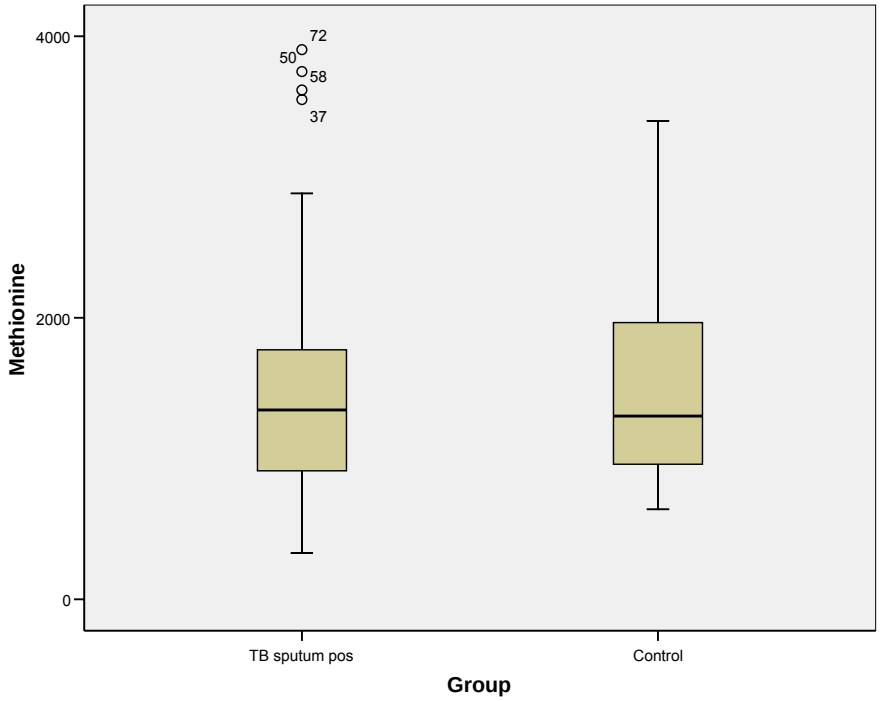

Figure 3.7: Box and whisker plot of mg methionine intake by TB patients and controls.

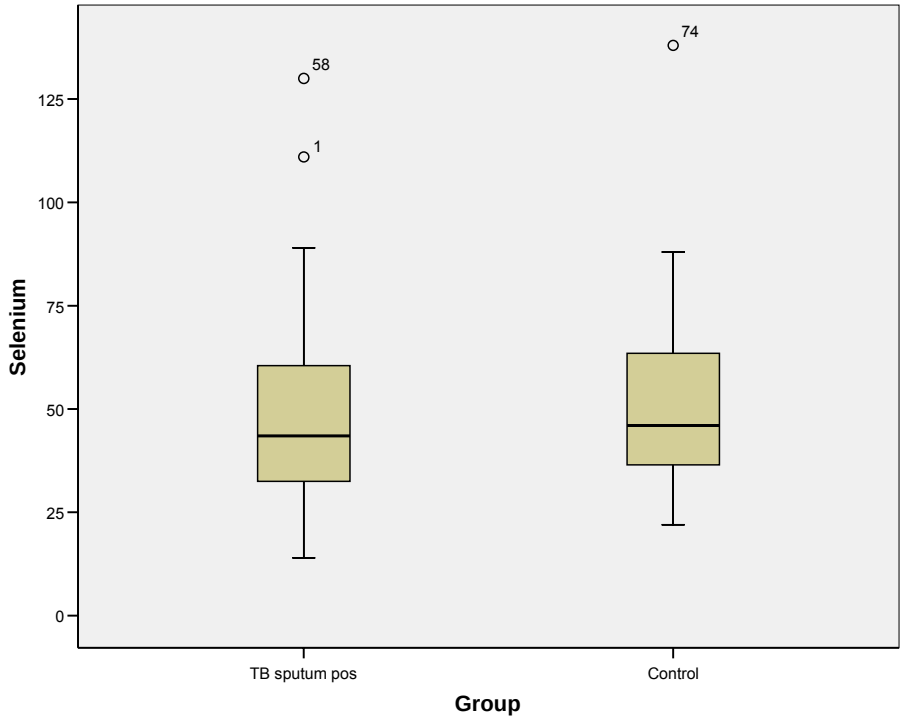

Figure 3.8: Box and whisker plot of µg selenium intake in the 2 groups.

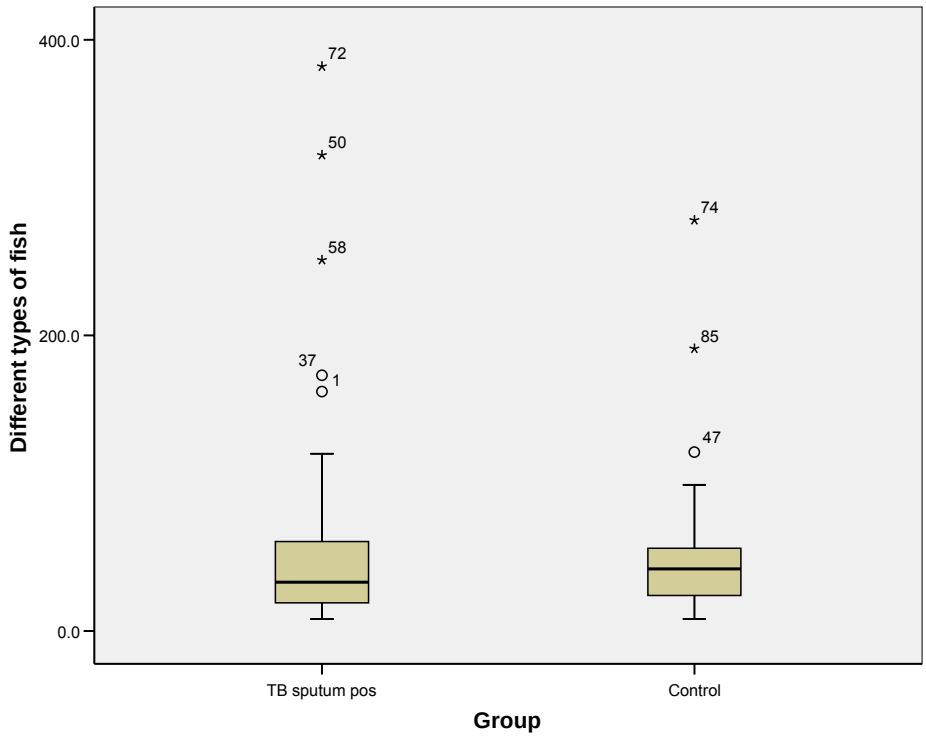

Figure 3.9: Box and whisker plot of gram intake of fish by the 2 groups.

Median, 25 and 75% quartiles and whiskers are visualised in the figures. The outliers are included in the analysis as they are probably based on fish intake.

3.3.7 Associations

Table 3.4 shows that there is a strong correlation between the intakes of methionine, cysteine and selenium, but no significant correlation between BMI and any of the amino acids or selenium. The correlation is slightly higher between the components in TB patient group than in control group.

Table 3.4: Correlation between intake and BMI

		methionine	cysteine	selenium
Controls	BMI	r = 0,13 P > 0,05	r = 0,11 P > 0,05	r = 0,22 P > 0,05
	methionine		r = 0,92 P < 0,01	r = 0,77 P < 0,01
	cysteine			r = 0,69 P < 0,01
TB patients	BMI	r = 0,06 P > 0,05	r = 0,03 P > 0,05	r = 0,21 P > 0,05
	methionine		r = 0,95 P < 0,01	r = 0,90 P < 0,01
	cysteine			r = 0,84 P < 0,01

3.3.8 Seasonal change

People mostly harvested in April 2006 and were heavily dependant on locally produced food. By unstructured observation most people only stored maize or rice and grained the maize every second week. Other food were collected or bought on a daily basis. During the study period there was a clear shift of seasonal fruits and vegetables. In August oranges and the fruit masau were widely available. As these declined in September you could see an increase of pawpaw and sugarcane.

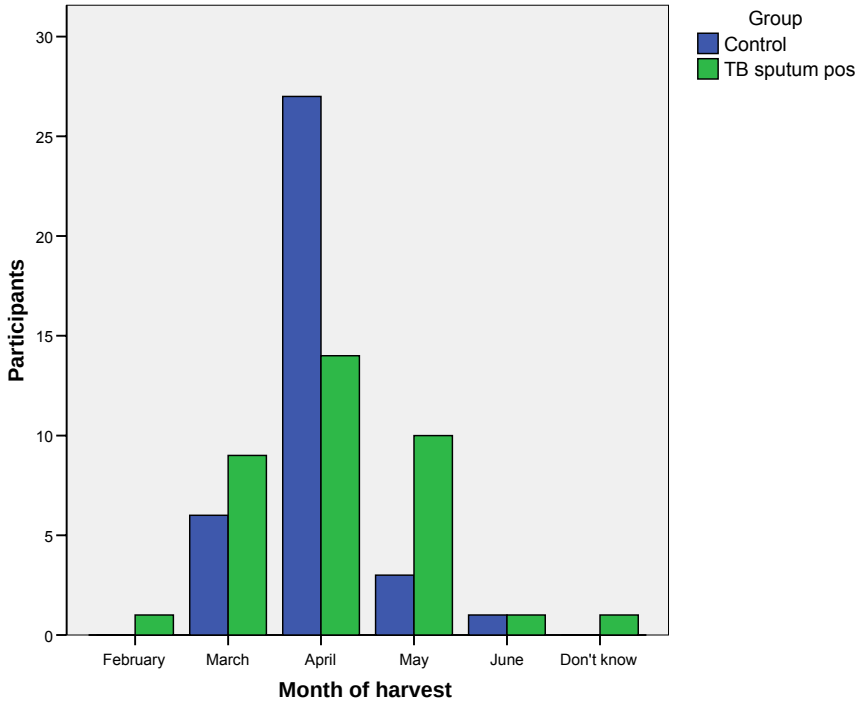

Figure 3.10: Participants reporting month of harvest

In October the mangoes started to get ripe and the intake of sugarcane dropped. Other seasonal fruits like masuku, gwafa, tende and avocado were eaten in small amount. The availability of vegetables like cassava and sweet potato decreased during the research period and are considered half-seasonal. The median duration for the crop to last in 36 TB patients households was 7 months (IR 2,4) as it was for 37 households of controls (IR 3,3). No significant difference was found. (P-value>0,05)

In Figure 3.11 the participants' selenium intake is shown during the study period which started beginning of September until beginning of December. Similar graphs were seen for

methionine and cysteine. No significant correlation was found between either of the components and date of intake with a significance level of 0,05. The range is larger in November than in September.

The TB patients did their 24 hour recalls in September(14), October(2), November(20) and December(4). The adult controls did them in September(9), October(3), November(22) and December(6). The distribution during the week is skewed but quite similar concerning the 2 groups. TB patients did interviews on Monday(1), Tuesday(11), Wednesday(13), Thursday(2), Friday(6), Saturday(3) and Sunday(4) and controls did interviews on Monday(1), Tuesday(13), Wednesday(12), Thursday(5), Friday(3), Saturday(2) and Sunday(4).

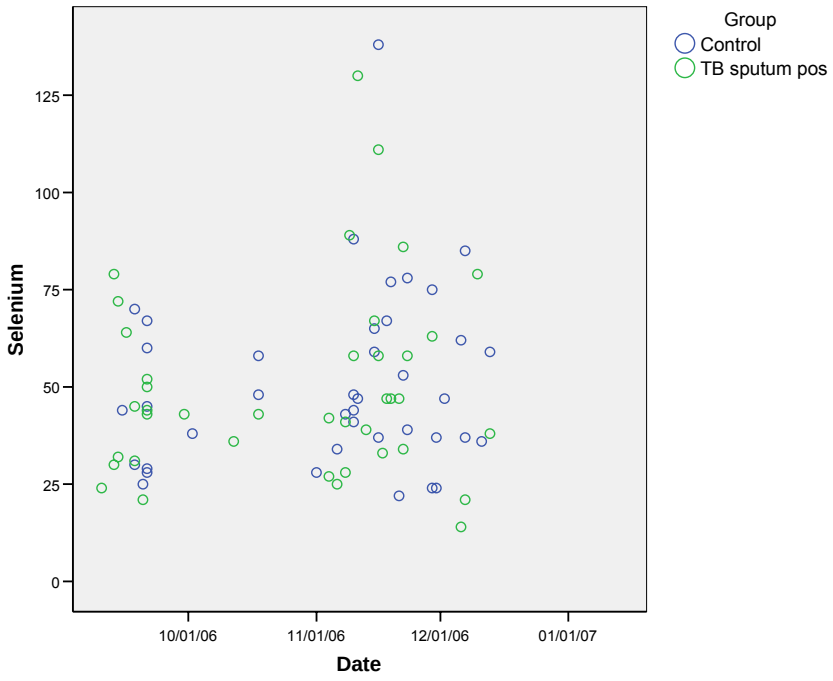

Figure 3.11: Selenium intake by participants distributed during the study period.

3.3.9 Illness

When the participants were asked if they were sick during the recording day 10/80 reported that they were sick. None of the 2 controls and only 3/8 TB sputum positive patients reported that the sickness affected their appetite. One control reported taking painkillers. 5 TB patients reported taking tablets, 3 reported taking TB tablets and 3 reported painkillers. There was no significant difference between the groups. (P-value>0,05)

3.3.10 Anti nutrients

3.3.10.1 Cyanogens

Cassava which is a contributor of cyanogens in the diet was eaten by 10/40 TB sputum positive patients and 8/40 controls. They had a median intake of 171 gram (IR 166) and 175 gram (IR 118) respectively. No significant difference was found with a significance level of 0,05. About half ate the cassava raw after removing the peel. Others cooked them and ate the root without graining.

3.3.10.2 Iodine

The kitchen salt sold unprotected from sunlight at the markets was iodinated. Control group had a median consumption of iodine of 236µg (IR 243) compared to TB patients who had a median of 253 µg (IR 292). No significant difference was found (P-value > 0,05).

3.4 Content of sulphur amino acids

Table 3.5: Protein and sulphur amino acid content in food items from Mangochi district

Chichewa name	**English name**	**Scientific name**	**Number of food items**	**Protein N x 6,25 (%)**	**Methionine (g/kg)**	**Cysteine (g/kg)**	**Remarks**
				`+/- 5%	**`+/- 5%A**	**`+/- 5%A**	
Method				EU DIR 93/28 m	AOAC 994.12 199 L	AOAC 994.12 199 L	
Nzima	Brown stiff maize porridge		1	2,8 +/-0,14	0,7 +/-0,04	0,6 +/-0,03	Cooked
Nzima	White stiff maize porridge		1	2,5 +/-0,13	0,6 +/-0,03	0,5 +/-0,03	Cooked
M'gayiwa	Brown maize flour	*Zea mays*	a	7,6 +/-0,38	1,5 +/-0,08	1,9 +/-0,10	Raw
Ufa woyera	White maize flour	*Zea mays*	a	7,8 +/-0,39	1,4 +/-0,07	1,8 +/-0,09	Raw
Usipa	Cyprinids	*Engraulicypris sandela*	>20	67,0 +/-3,4	18 +/-0,90	7,9 +/-0,40	Raw
Thobwa	Gruel		1	0,8 +/-0,04	0,1 +/-0,001	0,1 +/-0,001	Fermented
Nyemba	Kidney beans	*Phaseolus vulgaris*	a	21,2 +/-1,1	2,4+/-0,12	2,2+/-0,11	Raw

a. 3 heaps bought from 3 different vendors mixed.

3.5 Content of selenium

Table 3.6: Selenium content in selected food items from TA Mponda in Mangochi district.

No.	Chichewa name	English name	Scientific name	No of food items (a)	% water	Se µg/ 100g	Remarks
1	*Masau*	Catch thorn	*Ziziphus mauritina*	>20 (1)	78	0,9	
2	*Malambe*	Monkey bread fruit	*Adansonia digitata*	>20 (3)	11	1,4	Dried
3	*Mpunga*	Rice	*Oryza sativa*	>20 (1)	14	1,0	Dried
4A	*Anyesi*	Onion, white	*Allium ascalonicum*	5 (1)	92	1,1	
4B	*Anyesi*	Onion, red	*Allium ascalonicum*	5 (1)	88	5,4	b
5	*Nthochi*	Banana	*Musa paradisiaca sapientum*	3 (1)	78	0,9	
6	*Usipa*	Cyprinids (Fish)	*Engraulicypris sandela*	>20 (1)	14	84,2	Dried
7	*Mapira*	Giant millet	*Sorghum bicolour*	>20 (3)	13	8,4	Dried
8	*Mkwani*	Pumpkin leaves	*Cucumis metuliferus*	5 (1)	82	7,0	
9	*Chainisi*	Chinese cabbage	*Brassica chinesis*	10 (1)	92	0,2	
10A	*Mati mati*	Tomato	*Lycopersion esculentum*	12 (1)	96	0,8	c
10B	*Mati mati*	Tomato	*Lycopersion esculentum*	6 (1)	95	1,0	c
11	*Chinangwa*	Cassava	*Manikot esculenta*	9 (1)	59	<LOQ	
12	*Mbatata*	Sweet potato	*Impomea batas*	8 (2)	65	3,3	
13	*Mcheni*	Cichlids (Fish)	*Ramphochromis*	9 (1)	66	30,5	Smoked
14A	*Ufa woyera*	Maize flour, white	*Zea mays*	>20 (1)	12	1,4	d, Dried
14B	*Ufa woyera*	Maize flour, white	*Zea mays*	>20 (1)	17	6,5	d, Dried
15	*Ufa wa m'gayiwa*	Maize flour, brown	*Zea mays*	>20 (3)	16	4,9	Dried
16	*Utaka*	Cichlids (Fish)	*Copadichromis quadrimaculatus*	12 (1)	40	39,8	Dried
17	*Nkhungudzu*	Hyacinth bean	*Lablab purpureus*	>20 (2)	10	28,6	Dried
18	*Nandolo*	Pigeon peas	*Cajanus cajan*	>20 (1)	2	15,5	Dried
19A	*Madzi*	Borehole water	*Aqua*	1	100	0,018	c
19B	*Madzi*	Borehole water	*Aqua*	1	100	0,079	c

19C	*Madzi*	Borehole water	*Aqua*	1	100	0,030	c
21	*Mapapaya*	Paw paw	*Carica papaya*	3 (1)	91	0,2	
22	*Thobwa*	Gruel		>20 (3)	93	0,4	
23A	*Mtedza*	Ground nuts	*Arachis hypogea*	>20 (1)	0	11,3	c, Dried
23B	*Mtedza*	Ground nuts	*Arachis hypogea*	>20 (1)	8	7,4	c, Dried
24	*Nzimbe*	Sugar cane	*Saccharum officinarum*	1	70	<LOQ	
25	*Mango*	Mango	*Mangifera indica*	5 (1)	87	2,7	
26	*Mchere*	Salt		>20 (1)	2	<LOQ	e
28	*Therere*	Okra	*Abelmoschus esculentus*	>20 (1)	83	<LOQ	
29	*Mpiru*	Indian mustard/Turnips	*Brassica juncea*	10 (1)	90	11,7	
30	*Kobwe*	Cow peas	*Vigna unguiculata*	>20 (2)	3	16,3	Dried
31	*Nyemba*	Kidney beans	*Phaseolus vulgaris*	>20 (1)	14	2,2	e, Dried
32	*Ufa wa tirigu*	Wheat flour	*Triticum aestivum*	>20 (1)	14	7,9	e, Dried
33	*Nimu*	Neem	*Azadirachta indica*	(1)	12	15,1	d, Dried
34	*Kambusi*	Cichlids (Fish)	*Astatotilapia calliptera*	>20 (1)	24	40,9	Dried
35	*Nzima*	Maize porridge, brown		(1)	77	1,1	Cooked
37	*Nzima*	Maize porridge, white		(1)	77	1,5	Cooked
38	*Kale ofiyira*	Red curry powder	*Bixa orellana*	>20 (1)	12	10,5	Dried
39	*Mbirichila*	Yellow curry powder	*Bixa orellana*	>20 (1)	15	4,6	Dried
40	*Tsabola*	Pepper	*Capsicum annuum*	11 (1)	72	8,6	
41	*Chambo*	Tilapia/Cichlids (Fish)	*Onlochromis saka*	1	78	6,8	
42	*Mbatata kachwere*	Potato	*Solanum tuberosum*	10 (1)	81	0,2	
43	*Masamba a tiyi*	Tea leaves		(1)	12	17,9	d, Dried
44	*Mazira*	Chicken egg		4 (1)	75	15,0	
45	*Mafuta*	Vegetable oil		(1)		<LOQ	e

a - Number of households/vendors/gardens where food items are collected from

b - Selected value for food item used in calculation of intake.

c - Average value used in calculation of intake.

d - Food item not used in calculation of intake.

e – Not from TA Mponda

LOQ - the value is too low to tell with certainty.

Table 3.5 shows the seven selected food items analysed for sulphur amino acids from Mangochi district. The selenium values for 42 different food items are presented in Table 3.6. For some food items 2-3 analyses were done. Food item 4A and 4B had different colour. Food item 10A was collected from Katema area inland and 10B from Kafulumira area close to the lake. For food item 14A two fertilizers called Urea/Calcium Ammonium Nitrogen (CAN) without sulphur was used in the garden. This was not used for food item 14B. Water sample 10A and C is from Katumbiri and Mpinganjira respectively along the lakes. Sample 10B was inland from Katema area. Food item 23A was a ground nut type called CG7 and food item 23B was from a type called Chalimbana. They were not collected from the same area

3.5.1 Farming

Concerning the level of selenium in plants, the use of sulphur containing fertilizer and burning of the fields may have an influence. In Table 3.7 the use of fertilizer in the field is shown. More participants than the farmers (32 TB patients and 34 controls) answered the question about using fertilizer. One TB patient and two controls who were not farmers answered yes.

Table 3.7: Use of fertilizer by participants

			Group		
			TB sputum pos	Control	Total
Fertilizer in field	Yes	Count	25	26	51
		% within Group	73,5%	70,3%	71,8%
	No	Count	9	11	20
		% within Group	26,5%	29,7%	28,2%
Total		Count	34	37	71
		% within Group	100,0%	100,0%	100,0%

Of the 51 participants using fertilizer in their field over half of them used Urea and CAN. It was just minor differences between the two groups. 20% used just Urea and 14% used Urea and 23/21/04Sulphur. The rest used either CAN or 23/21/04Sulphur alone or both in combination with Urea or Dcompound. No significant difference between the groups was found. P-value>0,05

Table 3.8: Participants history of burning the field to clean it

			Group		
			TB sputum pos	Control	Total
Burning the field	Yes	Count	10	13	23
		% within Group	29,4%	35,1%	32,4%
	No	Count	24	24	48
		% within Group	70,6%	64,9%	67,6%
Total		Count	34	37	71
		% within Group	100,0%	100,0%	100,0%

There was no significant difference of practice between the two groups. (P-value >0,05) About 32% of the overall participants reported that they burn the field to clean it.

3.6 Body mass index

The TB sputum positive patients had a mean BMI of 18,6 (SD 2,38) compared to 20,7 (SD 2,49) in the control group. The mean difference showed to be -2,15 (SD 2,95)(Confidence interval (CI) -3,09/-1,21) with a high significance, (P-value < 0,001). Analysing for men and women separately showed that this significant difference continued for men with a mean difference of -2,63 (SD 2,42) (CI -3,73/-1,53). For women the mean difference was -1,62 (SD 3,43) (CI -3,28/ 0,03) and thereby not significant.

Table 3.9: Distribution of BMI in sputum positive TB patients

Severely thinness (< 16,0)	4
Moderate thinness (16,0-16,99)	4
Mild thinness (17,0-18,49)	13
Normal range (18,5-24,99)	18
Pre obese (25,0-29,99)	1
Total	**40**

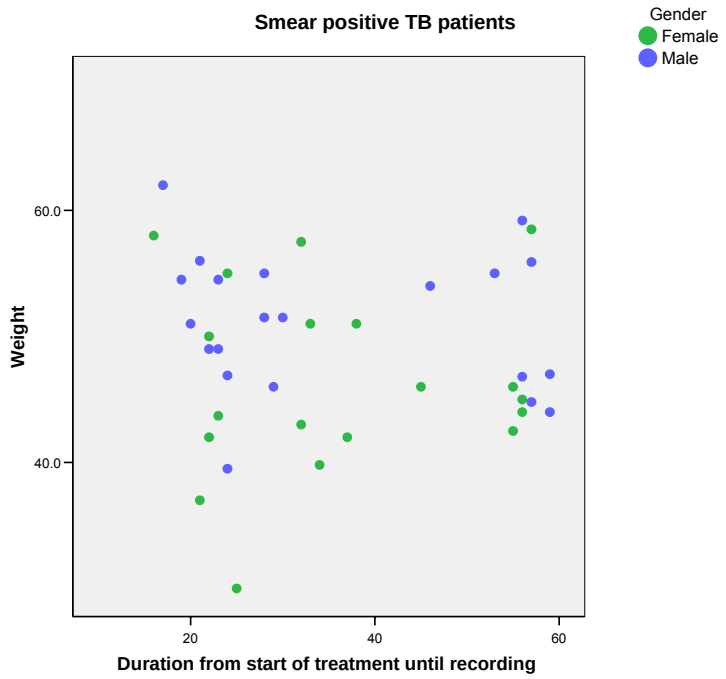

Figure 3.12: TB patients' weight related to duration of treatment at recording day.

In Figure 3.12 it seems one group had a generally higher weight than the other one, but TB patients recorded late during the 14 to 60 days of treatment did not have a significantly higher or lower correlation of BMI, r = -0,03, P-value > 0,05. A significant difference is not seen either when including duration of symptoms before diagnosis concerning either weight or BMI, r = 0,09, P-value > 0,05 as seen in Figure 3.13.

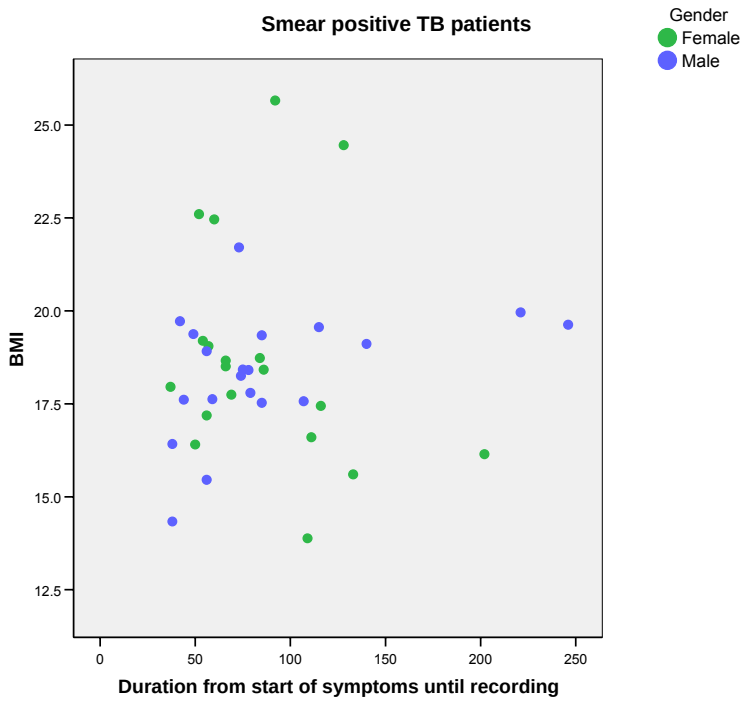

Figure 3.13: Distribution of TB patients' BMI to duration of cough at recoding day.

3.7 Risk factors

3.7.1 Risk of Tuberculosis

As there was no significant difference of intake in either of the nutrients between group of TB sputum positive patients and controls, it is interesting what may be a risk for being a TB sputum positive patient or control. Since there is no time event recorded a causal relationship can not be presented. In table both univariate analysis and multivariate was done to compare the results with or without confounding factors. Age, gender and village are left out because the study is individually matched. The indicator is set on the most common subgroup and the odds ratio is the relation between the subgroup and the indicator subgroup. As seen in Table 3.10 higher education has a significant preventive effect compared to none education, Odds ratio (OR) 0,02 (CI 0,0-0,67) with a P-value = 0,029 after adjusting for confounding factors. Living in a house with wood or cement floor is a significant risk compared to living in a house with sand/dung or earth, OR 11,8 (CI 1,03-134) with a P- value = 0,047.

Table 3.10: Risk factors for having smear positive tuberculosis

			Univariate analysis			Multivariate analysis		
		Number	OR	95% CI	P-value	OR	95% CI	P-value
School years	None	24						
	Primary 1-4	17	1,37	0,44-4,28	>0,05	2,57	0,48-13,8	>0,05
	Primary 5-8	20	1,24	0,38-4,03	>0,05	1,53	0,30-7,92	>0,05
	Secondary or higher	18	0,43	0,10-1,78	>0,05	**0,02**	**0,00-0,67**	**0,029**
Assets	Very Poor	36						
	Poor	28	0,90	0,28-2,92	>0,05	1,77	0,22-14.4	>0,05
	Fair	16	1,39	0,31-6,29	>0,05	3,24	0,19-54,8	>0,05
Animals	Yes	39	0,44	0,14-1,44	>0,05	0,46	0,10-2,08	>0,05
	No	41						
Occupation	Subsistent farmer	39						
	Non Farmer	14	2,30	0,63-8,44	>0,05	10,9	0,82-146	>0,05
	Farmer and other	27	2,59	0,94-7,17	>0,05	3,49	0,64-18,9	>0,05
Flooring	Sand/dung or earth	61						
	Wood or cement	19	2,00	0,68-5,85	>0,05	**11,8**	**1,03-134**	**0,047**

These results may be shown in a cross tabulation comparing the raw data in the two groups. In Table 3.11 it is shown that fewer of the TB sputum positive patients had higher education than the controls.

Table 3.11: Number of participants who started education.

			Group		
			TB sputum positive	Control	Total
School level	None	Count	12	12	24
		% within Group	30,8%	30,0%	30,4%
	Primary 1-4	Count	10	7	17
		% within Group	25,6%	17,5%	21,5%
	Primary 5-8	Count	11	9	20
		% within Group	28,2%	22,5%	25,3%
	Secondary or higher	Count	6	12	18
		% within Group	15,4%	30,0%	22,8%
Total		Count	39	40	79
		% within Group	100,0%	100,0%	100,0%

Comparing literacy towards education figure shows that all with an education of 5-8 years reported they could read. Still about 60% of the ones with an education of 1-4 years could not read.

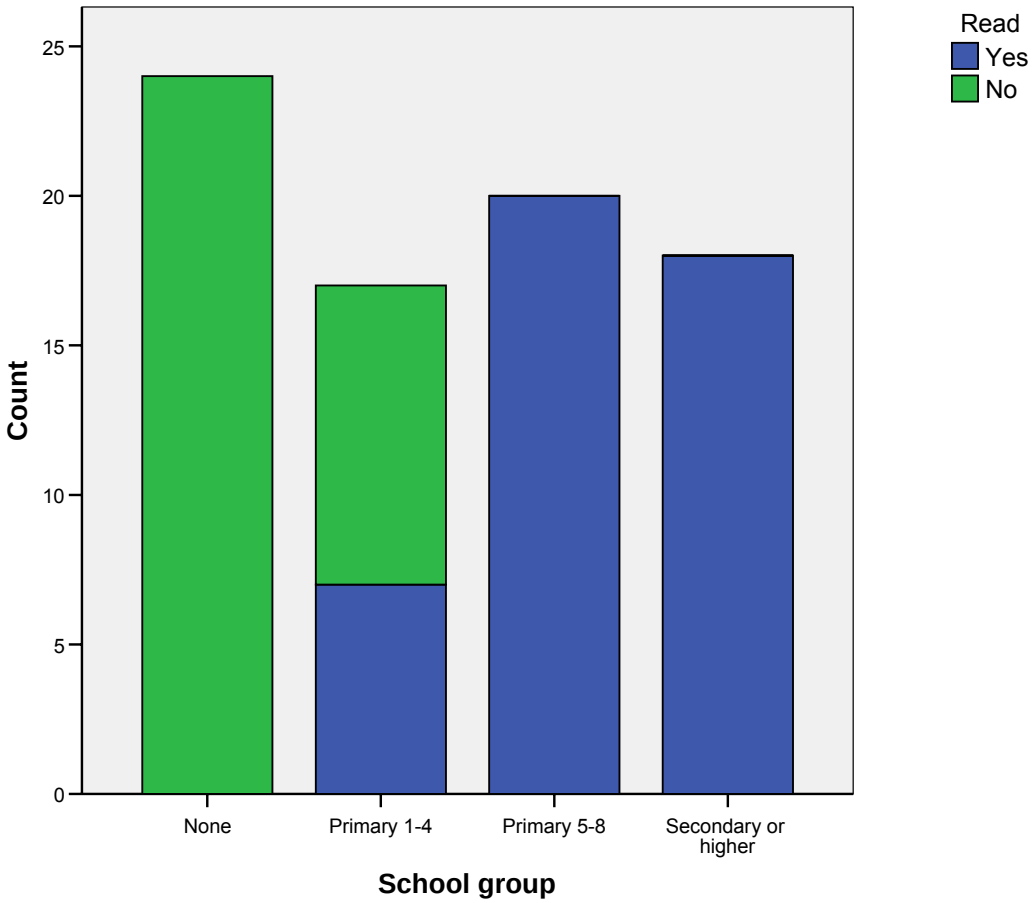

Figure 3.14: Proportion of participants who could read or not divided in school groups.

Risk of one day deficient intake

Following WHO's Recommended Nutrient Intake (RNI) for Selenium < 34 µg/day for men and < 26 µg/day for women, methionine < 676 mg/day and cysteine < 676 mg/day (13mg x 52kg x 1, where 52 kg is the mean weight of the participants) the proportion of participants who had a one day deficient intake is presented in Table 3.12 (41;42).

Table 3.12: Number of participants who had a one day deficient intake.

			TB sputum positive	**Control**	
One day deficient intake	**Selenium**	Yes	7	6	13 (16%)
		No	33	34	67
		Total	40	40	80
	Methionine	Yes	2	1	3 (4%)
		No	38	39	77
		Total	40	40	80
	Cysteine	Yes	8	7	15 (19%)
		No	32	33	65
		Total	40	40	80

3.7.2 One day deficient selenium intake

Dividing the 80 participants into 2 groups where 13 of the participants with a one day deficient selenium intake are in one and 67 of those with a one day sufficient intake are in the other one, it is interesting to see if there are any risk factors of having a deficient selenium intake. These groups are not matched. The indicator is set on the largest subgroup for categorical variables. Univariate and multivariate analysis is shown in Table 3.13 to compare with or without confounding factors. Rising age is the only significant risk factor of having a deficient selenium intake in both univariate and multivariate analysis. OR 1,08 (CI 1,001-1,17) P-value = 0,046.

Table 3.13: Risk factors for having a one day deficient selenium intake

		Number	Univariate analysis			Multivariate analysis		
			OR	95% CI	P-value	OR	95% CI	P-value
Gender	Female	38	1,97	0,59-6,67	>0,05	3,32	0,69-16,0	>0,05
	Male	42						
Age		80	**1,07**	**1,003-1,14**	**0,04**	**1,08**	**1,001-1,17**	**0,046**
School years		79	0,90	0,77-1,04	>0,05	0,94	0,78-1,13	>0,05
Assets	Very Poor	36						
	Poor	28	1,35	0,35-5,21	>0,05	2,63	0,55-12,5	>0,05
	Fair	16	1,43	0,30-6,89	>0,05	3,25	0,46-23,0	>0,05
Animals	Yes	39	0,88	0,27-2,91	>0,05	0,37	0,08-1,65	>0,05
	No	41						
Occupation	Subsistent farmer	39						
	Non Farmer	14	0,65	0,12-3,49	>0,05	1,56	0,17-14,6	>0,05
	Farmer and other	27	0,48	0,12-2,02	>0,05	0,55	0,10-3,03	>0,05
Flooring	Sand/dung or earth	61						
	Wood or cement	19	0,23	0,03-1,87	>0,05	4,48	0,36-55,4	>0,05

3.7.3 One day deficient cysteine intake

If dividing the participants in two, where 15 had a one day deficient intake and 65 a one day sufficient intake, there may be some risk factors for ending up in either of the groups. Table 3.14 shows the results of univariate and multivariate analysis and age is a significant risk factor for a cysteine deficient intake. OR 1,09 (CI 1,02-1,18) P-value = 0,019 No other demographic or socioeconomic factor tested for were a significant risk. (P-value>0,05)

Table 3.14: Risk factors for having a one day deficient cysteine intake

		Number	Univariate analysis			Multivariate analysis		
			OR	95% CI	P-value	OR	95% CI	P-value
Gender	Female	38	1,86	0,59-5,84	>0,05	3,39	0,74.15,6	>0,05
	Male	42						
Age		80	**1,08**	**1,02-1,15**	**0,014**	**1,09**	**1,02-1,18**	**0,019**
School years		79	0,88	0,76-1,02	>0,05	0,98	0,82-1,18	>0,05
Assets	Very Poor	36						
	Poor	28	2,33	0,44-12.3	>0,05	0,63	0,14-2,85	>0,05
	Fair	16	1,17	0,19-7,21	>0,05	0,96	0,13-7,25	>0,05
Animals	Yes	39	0,65	0,21-2,03	>0,05	0,27	0,06-1,16	>0,05
	No	41						
Occupation	Subsistent farmer	39						
	Non Farmer	14	0,26	0,03-2,24	>0,05	0,52	0,04-6,24	>0,05
	Farmer and other	27	0,76	0,22-2,58	>0,05	1,09	0,24-5,07	>0,05
Flooring	Sand/dung or earth	61						
	Wood or cement	19	0,19	0,02-1,52	>0,05	0,21	0,02-2,52	>0,05

4 Discussions

4.1 Methodological discussion

4.1.1 Strengths

Strength of the diet interview of this study is the use of an already validated method in a similar population. At the same time no participants reported an unusual diet and only 3/80 reported that it had been a feast day. This strengthens the assumption that this study gives a true picture of the normal diet. Selenium intake is based on local values which eliminate geographical variation. A strength of a matched study is that the between subjects variability is removed. The within subjects difference, or here the difference between TB patients and controls is then the focus.

4.1.2 Weaknesses

The sample is small and smaller than the calculated sample size. As the main null hypothesis is not rejected the possibility of confirming the null hypothesis even if there is a difference is present. The probability of type 2 error is high. The small sample will as well influence the strength of other results and has to be interpreted in this perspective.

4.1.3 Researcher's bias

The researcher has his prejudices and expected to find a difference of intake in the two groups and to link TB to poverty in the Malawian society. During field work the participants' diet seemed very much alike and a result showing no significant difference of intake was seen as more interesting. Heidi Arntsen found as well a significant difference in selenium serum levels before selenium intake was calculated. There is a clear advantage one researcher following all steps in a study as it is a disadvantage. The closeness to the data may blind the researcher of overall perspective but is an advantage as he/she has in dept knowledge. No conscious actions have been taken to influence the results in a specific direction but the role of the prejudices may not be ignored.

4.1.4 Selection

Most of the patients lived in TA Mponda and TA Chowe. As recruitment of TB patients were done by the research teams at the two hospitals in the respective TAs, the distance to these hospitals may be a selection factor. There are probably two main reasons, either that the patients were not able to come to the hospitals or that the researchers did not reach the patients.

The treatment was free of charge but the relatives had to provide food for themselves and some to the patient. Some TB sputum positive patients may not been able to come to the hospitals because of their bad health, lack of transportation or funding. Of a population of approximately of 600 000 and a smear positive incidence rate of 183/100 000 population/year it should be about 92 new cases per month (8;12). The two hospitals in Mangochi district admitted about 30 new smear positive patients per month together.

33 of 102 TB sputum positive patients admitted to the hospital were not enrolled. These were mainly patients who had been discharged after 1-14 days of hospitalisation and lived far away, getting their treatment from a local health centre. As actual study was done together with another study collecting blood and there was a 40 minute time limit before preparing these samples, living far meant exclusion. 2 did as well die at arrival. A few of them were too old and got excluded.
Living far from the hospitals would sometimes mean living far from the lake. Living far could have a negative influence on intake of fish and therefore the intake of selenium and sulphur amino acids. As the participants were matched this would probably not have an influence on the main result if the patients and controls differ more in the rural that in central Mangochi. The participants living in rural areas of the district do not indicate this.

There was a low response rate in this study. The reason was probably because the participants were asked to join another study at the same time where they were asked to donate blood. Many were suspicious to donate blood and showed discomfort. Assumingly many were afraid the blood should be tested for HIV without their consent. The researchers were also on one occasion asked if they were Satanists, called vampires and almost chased from one village. In addition 2 died after recruited. Of the 11 rejecting and 14 not following through several complained of their bad condition. This may indicate that the worst affected was selected out.

The controls were found through a random walk method. This was based on the knowledge of the village chief who showed where the village centre was. There are no indications that any of the village chiefs mislead the researchers on purpose, but the accuracy may have differed from one village to another. The TB patients were selected because diagnosed with smear positive TB. Because the controls were matched to the TB patients they may not be representative for population in Mangochi. This was not the aim either. Obviously the average age is higher in participants than in the general population as inclusion criteria was age between 15 – 60 years.

4.1.5 Criteria and matching

Due to limited time and funding, a second Ziel-Nielsen staining was not done. Even if the hospitals had trained laboratory technicians, there could be patients diagnosed with pulmonary TB who did not have it. With a limited sample size this could have an effect on the result.

As some of the villages were part of a bigger village, a mistake was done and one participant from Mapira got a match from Mbaluku. This should not have any relevance as they are both a part of Mangochi town. By unstructured observation people were using the same markets and having the same socioeconomic standard.

4.1.6 Sample size

The samples size was based on the assumption that 70 % of the TB sputum positive patient would have a deficient selenium intake based on studies in Africa which shows 67-83% of study population had a deficient intake (33;43). Calculating deficiency rate from present study is not justified but it may give an indication of the true level. As seen in Table 3.12 7/40 TB patients had a one day deficient intake or 18%. With this low deficiency and that a constant odds ratio, confidence level and power and a confidence interval not including 1, the sample size had to be 300 with 150 TB patients and 150 controls. It isn't. As the aim is to describe the situation before an eventually nutrition intervention, you could argue that the difference had to show with this sample if an intervention could be justified.

4.1.7 Interview method

The interview method used was taken from the manual "An Interactive 24-hour Recall for Assessing the Adequacy of Iron and Zinc Intakes in Developing Countries" (38). The method was validated with Malawian women from Mangochi district for energy, protein, fat, fibre, phytate and minerals (Fe, Cu, Ca, Mn, Zn). Ferguson's study concluded that the median daily intakes of most minerals recorded with the actual method were comparable to the median intakes from a weighed record. Recalled protein and fat intakes were significantly underestimated compared to weighed intakes. The variance ratio (intra variance/inter variance) was greater in recalled data than the weighed. Recalled data showed also underestimation of portion sizes of staple food and an overestimation of portion sizes of relishes but maize porridge and fish were within $\pm$15% of the median percentage ratio (44).

By comparison about half of the participants in this study were female. In a household mainly women cooked the food and would therefore assumingly have a better knowledge about what they ate. Contradictory to this the interviews with men did not indicate less accuracy. A main reason may be that the amount of ingredients in the relishes was calculated by standards and not by interview. The population should therefore be comparable.

Not all food items were shown in the picture charts. The pictures were chosen of what food items believed were common eaten. This could lead to an underestimation of food items not shown. Except water, milk powder, banana cake, bread and several other food items in Table 3.3 were not indicated in the picture charts. Less than 5 people ate these food items each. It is believed that the picture chart fitted the common diet well but presenting selected food items may skew the outcome.

Fish represented about 1/3 of the selenium intake in both TB patients and controls and people generally ate fish in a relish together with maize porridge. Ferguson's study comparing recall and weighed record found an overestimation of relish in recalls, which may lead to an overestimation of selenium intake. Stiff maize porridge was generally underestimated, and represented in this study ¼ of the selenium intake. The underestimation of maize has to be lager as the content is lower to equalise the overestimation of fish, which it slightly is. The over- and underestimation should also affect other minerals which have high concentration in green vegetables and beans which are the most common relishes. This is not seen in the study's conclusion and would probably count for selenium as well.

For protein intake, Ferguson's study showed a significant difference. The actual difference was 6,6 gram protein or 12% of the total weighed median. The main objective in this study is not to assess the proportion of deficiency in a population but comparing the intake between two groups. A small error would affect both groups and probably not the comparison of sulphur amino acid intake much.

Selenium and sulphur amino acids have a high concentration in some food items but their distribution is not different from Zinc and Iron. The within subject- and between subject variability is higher when a vitamin or mineral is concentrated in few food items than a vitamin or mineral with a wide distribution. The within subject variability is generally larger than the between subject variability for most nutrients. This means that the mean intake of a group may be assessed with a better accuracy than the mean individual intake (38). A study from Malawi assessing the intra/interindividual variance found that within subject variance was higher in developing countries than in developed countries. The reasons given were higher seasonal variances due to different food availability. They especially found a high within subject variance in micronutrient intake (45). This is based on a true yearly intake and an intake with a narrower time limit would therefore need less record days. In this matched study the between subject variability is removed and the within subject variability is the variability between TB patients and controls. As the variability is looked upon on a group level and not for each individual pair the method could be justified.

4.1.8 Interview bias

The student researchers had white skin, spoke English only and were regarded as rich foreigners. The groups arrived in most villages by car which only few Malawians could afford. The high socioeconomic status of the student researchers and the 2 female research assistants may have an influence of the answers given by the participants. As many of the participants were poor in comparison, it is likely that some felt discomfort of giving information about diet and socioeconomic status and they may have overestimated the answers.

When the foreign researcher visited, many people and especially kids were curious and wanted to see what was going on. Even if the researchers tried to put a strict policy on who

could listen to the 24 hour recall, it was not always possible to be alone with the participant. Most of the interviews were held outside with only a thin fence between private and public space. This may have an affect on the answers given but it is unlikely to have a major impact as there were few of these interviews with listeners. The socioeconomic interview part was held confidential and the participant was always alone with the researcher and the research assistant.

In contradiction to this doubt of bias the research groups got three times the offer to eat food with a participant. This could show that the participants were not embarrassed over their food and open concerning their eating habits. The interviews were performed at the participants' home where they could feel confident and safe. The researchers showed respect towards the participant and tried to enable a safe environment where information could be shared.

4.1.9 Distribution of interviews

Even if the researchers tried to avoid it, an uneven distribution of interviews developed on both week basis and month basis. As this study is done from September until December, it could mean the results were misleading if the 24 hour recalls were done in the beginning of the research period for one group and in the end for the other. Looking at the data in results shows that the distribution is quite similar in the two groups. Muslim participants were not interviewed during Ramadan when they were fasting during daytime and ate at night. Patients are allowed to eat during daytime but their diet may be influenced by relatives eating schedule. As it was an individually matched study it was therefore best to avoid interviewing both Muslim cases and controls during this period. The over representing of interviews in Tuesdays and Wednesdays could affect the result in over representing of a common or special diet. As Mangochi was predominately Muslim, Friday was prayer day. The population of Malawi is generally Christian so Sunday was the public day off and the day for an eventually feast. This indicates that no special diet was overrepresented but may be underrepresented. During the weekdays no regular difference in diet was seen but it still may have an influence.

4.1.10 Calculation of intake

4.1.10.1 Data error

Using excel sheet to calculate the intake has the disadvantage of being a heavy work load and therefore higher possibility of plotting error. These errors will still be accidental and not systematic as they would be in a software programme. The automatic calculations in the excel sheet which could be systematic errors are checked and found reliable. Accidental error is also checked but may have been passed.

4.1.10.2 Raw vs. cooked values

The calculation of both selenium and sulphur amino acids intake is based mainly on raw food. Preparation of food by frying, boiling or fermenting may have an effect on the content of the component and thereby the intake. Most food eaten in Mangochi was cooked. The main meals consisted of cooked porridge and cooked relish. Snacks eaten between the meals were also heated like fried ground nuts or boiled or roasted maize cobs. Fruits were eaten raw and sometimes raw cabbage together with fried potatoes.

Looking the content of selenium in maize flour and maize porridge in table it may seem that boiling causes a loss of the mineral. Comparing the dry weight values in Table 4.1, there is about 15-20% less selenium in boiled porridge compared to flour. The analyses are done in 4 different samples. This estimate does not correspond with a study done where they heated and boiled different food. Cooked cereal and baked fish did not show any selenium losses but heating overnight at 100°C the selenium content in cereal reduced with 7-23% (46). Vegetables and dairy products have been reported to loose 50% of the selenium content by cooking (4). In this study all food samples were dry heated at 105°C before analysing which could mean that all food items had a slightly higher content than measured. The participants' second biggest selenium intake came from eating stiff maize porridge. Intake from stiff maize porridge is measured from cooked food and would mean the result is not that affected by an eventually error. Even if there is a small loss of selenium during processing the bioavailability could be increased and compensate for the loss (47). Relishes were heated before eating. When preparing the relishes, added water is not poured out after cooking the food. This means that if selenium is not evaporating with the steam or get destroyed by heating, it will be eaten.

Table 4.1: Comparing selenium values in raw and cooked food.

	µg/g dry weight		µg/g dry weight
Maize flour, white	0,079	Stiff maize porridge, white	0,063
Maize flour, brown	0,058	Stiff maize porridge, brown	0,049

Proteins are the major food component which is most reactive after some vitamins. Methionine and cysteine are 2 of the 4 most reactive amino acids and may be oxidized or react with other food components. The most known is Maillard reaction where proteins react with reducing sugars (48). Reacting with other components does not mean that the content is reduced but the sulphur amino acids may not be utilized as well. This issue will be discussed later. Comparing the local analyses of sulphur amino acids from maize flour and stiff maize porridge combined with the % of water calculated from the selenium samples, the values before and after cooking is not that different. The values are from 4 different samples and give only an indication that the content of sulphur amino acids is not reduced in maize porridge after cooking. No other ingredients were added in the porridge than fresh water and salt.

Table 4.2: Comparing sulphur amino acid values in raw and cooked food.

	mg/g dry weight			mg/g dry weight	
	Methionine	Cysteine		Methionine	Cysteine
Maize flour, white	1,7	2,2	Stiff maize porridge, white	2,6	2,2
Maize flour, brown	1,8	2,3	Stiff maize porridge, brown	3,0	2,6

4.1.10.3 Selenium analyse method

There are several methods to measure the level of selenium in food samples. In this study an inductively coupled plasma mass spectroscopy was used. One analysis was done of a composite sample with varying number of food items. Mean and SD of the reference material is showed below. Compared to uncertainty in other measures this uncertainty is small.

Reference material		**Selenium**
NCS DC 73348		
Reference value	µg/g	0,184±0,013
Mean	µg/g	0,180
Standard deviation	µg/g	0,004
DORM-2		
Reference value	µg/g	1,40±0,09
Mean	µg/g	1,364
Standard deviation	µg/g	0,011

Detection limit	(in dry weight)
Detection limit for 0,5g sample	0,002
Standard error for 0,5g sample	0,003
Quantifying limit	
Quantifying limit for 0,5g sample	0,011

A study analysing iodine and selenium both by neutron activation and by atomic absorption spectrophometry found up to 15% lower values in samples analysed with neutron activation than with atomic absorption (49). This shows that there might be difference in selenium level according to analysing method.

4.1.10.4 Sulphur amino acid analyse method

A method has always its advantages and disadvantages. One of the disadvantages of measuring amino acids is its high cost and need of sophisticated equipment. The result of this is only one analyse per composite food sample which lead to a higher uncertainty concerning the representability of result. Still the results from Mangochi correspond well with other food composition tables from the South Africa and Kenya. The advantage of the method is that it measures the direct amount of sulphur amino acids without dealing with nitrogen content, conversions factors and relative proportions. It is the method preferred by United Nations' Food and Agriculture Organisation (50).

4.1.11 Correlation and risk assessment

The individual diet can not be determined by one 24 hour recall. Still this is the basis of the risk assessment of one day selenium or cysteine deficient intake. The results are analysed on group level but some of the groups are quite small. One day 24 hour recall is also the basis when it comes to correlating intake with season and BMI. Here the one day individual intake is plotted through out the study period. As discussed before there is a high within subject variance in micronutrient intake. Nyamboses study estimated that 95-213 record days using weighed records were needed with an error of $\pm$ 20% to measure a true average individual intake (45). This is not the case in this study and the risk assessment and seasonal correlation from Mangochi have to be seen in this perspective.

The validity of the socioeconomic factors is difficult to assess. Owning animals do not differentiate between type and number of animals which are considerable factors. Asset variable does not either differentiate between types of asset but it is believed that some assets are naturally bought before others. It may not be like this. Type of flooring and school years are more descriptive. When dividing school years into school groups it was because of the

distribution natural to divide primary education into 2 groups as this was done in earlier literature (7).

4.2 Discussion of the findings

4.2.1 Characteristics

The TB sputum positive patients had a mean age of 33 years. This is lower than the mean number of 15 and 60 which is 37,5 years. TB is associated with poverty and vulnerability, something 30 year olds generally are not. Reasons for this could be that Malawi has a young population and there are more 20 year olds than 50 year olds which is true. A factor could be that younger population is probably more open to participate in a study than older and a natural selection will occur. As earlier studies have shown that about 70% of TB patients have HIV co-infection this could have an impact on the distribution of TB in the population. Half of the patients were women which correspond well with the general feeling that there were as many female patients as male in the hospitals.

4.2.2 Socio economy

The socioeconomic characteristics are quite similar between the two groups. Still when 16% of the participants reports that they do not have shoes and ⅔ do not have a mattress, it indicates that the general population is poor which corresponds with earlier findings. High number of the participants reported that farming they were doing farming understating the dependence on subsistence farming and consumption of locally grown food. The illiteracy level is high and slightly higher than the general characteristics of the rural population in Malawi in 2004(1). Women have also a higher illiteracy rate than men as found earlier (1). Compared to the findings from the study in Lungwena area the socioeconomic characteristics like sanitation, source of drinking water and literacy are generally better in this study from Mangochi district (7). This may be because of the proportion of urban participants in this study, gender or age distribution or other geographical variations. Half of the participants had a radio and about 1/10 a television. Together with literacy rate these findings give useful knowledge on how to reach the population with health information in a nutrition intervention.

4.2.3 Intake

No significant difference in selenium, cysteine or methionine intake was seen by TB sputum positive patients and matched controls. This could be due to different reasons. Obviously the sample size is low but the result indicates that the difference may not be large anyway.

Few of the TB patients reported that they were sick and that the sickness affected the appetite. As already discussed there may be a selection of TB sputum positive patients excluding the worst affected. If so it would probably influence the result. Another influence could be that many of the participants were interviewed at a late stage of their treatment. The participation included TB patients on treatment from 2 weeks until 2 months and half of the TB patients had been on treatment for a month before being interviewed. Outliers were seen in both selenium and methionine intake but also fish intake. The box and whisker plots showed that the medians, quartiles and whiskers were comparable.

In total the two groups had similar type of diet and only minor differences in amount. This study did not asked for local beliefs on which diet to eat when feeling sick but does probably not influence much. The common eaten food found in this study corresponded well to earlier findings in the same district (34). Even so, controls have a significant higher intake of stiff maize porridge but a significant lower diversity than TB patients even if they were eating as many times as them. This corresponds well with the idea that sick people eat less of untasty staple food and more of tasty relishes and snacks if they can afford. The higher diversity was seen in different type of food like cereal, legumes and vegetables but also in fruit type and snacks. A high diversity is favourable and could be good concerning protein quality in diet (31). It is also probably good for other nutrients not counted for in this study.

Fish, maize and mango were important sources of selenium intake. The picture was a bit more diverse for sulphur amino acid intake. Maize was the main contributor and fish, cereals, legumes and ground nuts were good sources. TB patients tended also to eat more rice than stiff maize porridge compared to controls. If this is due to lack of maize or a deliberate choice to vary the diet, is hard to tell. Rice had lower content of selenium and about similar level of sulphur amino acids as maize flour. It is also seen that both type of participants eat often food that do not contribute to selenium or sulphur amino intake, food like tomatoes, onions, sugar, salt and vegetable oil, while food like turnips, meat, milk and poultry with higher content is rarely eaten. This does not mean that intake of tomatoes or onions are irrelevant as they may contain other important nutrients. A general finding is that the interquartile range or range of intake is higher for TB patients than controls. This seems quite natural as the condition of sick people and their appetite may vary and therefore represent a wider spread than in a healthy population.

A study assessing intake of selenium of children 4- 6 years old in Malawi found that 43 % had an intake below the RDA of 20µg/day. The four main food sources of selenium were cereal products (35-60%), fish (20-27%), legumes (10-28%) and fruit and vegetables (5-11%). Meat and poultry contributed to less than 5% of the selenium intake (36). These numbers correspond well with the findings in this study. Fish has an even more central role in the diet in Mangochi than in Zomba which is natural as the Mangochi district surrounds southern Lake Malawi. Since fish has a higher content of selenium than other relishes like legumes or vegetables the intake will probably be higher in areas where fish is accessible. This could mean that people living far from the lake have lower selenium intake than those living close. A study from Burundi found that freshwater fish contributed from 16 to 50% of the selenium intake in different population. Middle class men and urban women had an intake of 82µg/day and 38µg/day respectively. The rural population who did not eat much fish had lowest intake with 17µg per day (32). These findings correspond well with the findings in this study even if geographical distribution of intake is not investigated due to limited information. The median intakes for the two groups in Mangochi were 44 and 46 µg/day and are considered as low (42). In addition the participants had an adequate intake of sulphur amino acids.

Adults have a proportionally higher intake of maize than children and it is natural that fewer have an intake less than RNI. Having one main source of a nutrient represents a danger if the crop fails. The selenium intake could decrease to about ¾ and the methionine and cysteine intake with down to ½. As the median intake of selenium was about 45µg, of methionine about 1,300g and of cysteine 0,922g this would probably be reduced to less than RNI of 13mg/kg/day. If the stiff maize porridge is not replaced with other staple food, the seasonal changes in selenium and sulphur amino acids intake would probably be larger when relying on maize as a main contributor.

Meat and animal products contributed proportionally little to the selenium and sulphur amino acid intake in this study as well. Controls ate more meat than TB patients who drank more milk. Meat is heavier to digest compared to milk and could be the reason for TB patients choosing cow milk instead of cow meat. Both food items have a high protein quality (31). Meat is probably more expensive but no other indicators suggest that controls were wealthier than the TB patients. It was not a significant difference of owning animals either between the groups. It may be added that milk and meat selenium values represented South Africa and as

content in animals varies along with geographical variation the content may be different in animals and animal products grassing in Mangochi district.

Vegetables did not contribute much to the intake of either selenium or sulphur amino acids. The most common vegetables eaten were tomatoes and onions but they contributed proportionally little to the intake of selenium and even less to intake of sulphur amino acids. Of green vegetable, Chinese cabbage were the one most common but had much lower selenium content than Pumpkin leaves and Turnips with quite high content. This could mean that there is a possibility of increasing the selenium intake if Chinese cabbage is replaced with Turnips of Pumpkin leaves in the diet. Economically this should work as the price at the market is similar. For sulphur amino acids the difference was little between the different vegetables.
Increasing selenium intake could also be achieved through eating more hyacinth beans, cow peas or pigeon peas which have a higher content of selenium than kidney beans which have a rather low content.

Water had low level of selenium and contributed little to total. The participants were drinking less than a litre per day. This is probably too little in an area with a day temperature 35-40 degrees C. The availability was high as seen from the characteristics collected where 64/80 reported less than 15 minutes to get water to a water source. Tea was consumed of the majority and tea leaves had a high level of selenium. This was not included in calculating selenium intake as it was difficult to estimate the dilution of selenium from the leaves to the water. This could mean that tea drinking was a source of selenium not counted for.

The intake of selenium, methionine and cysteine had a strong and significant correlation. Selenium could be in different chemical forms in food, inorganic forms like selenite, selenate or organic forms like selenmethionine (4). Sulphur amino acids are associated with protein rich food (30). A strong correlation could suggest that the selenium found in the food is mostly in organic form incorporated into proteins. This corresponds well with earlier knowledge that over 50% of selenium content in a plant can be in form of selenmethionine (25). A more obvious reason is that fish and stiff maize porridge is the two main contributors of selenium and sulphur amino acid intake. With such a large amount of the intake coming from two food types the mineral and amino acid may also correlate.

4.2.4 Seasonal changes

The results shows that over half, 42/80 participants did their 24 hour recall in November. With an over representing like this for one month it will probably shadow the other months and a monthly change in intake would be hard to see. This may be the reason for that a correlation between interview-date and selenium intake is not seen even when a seasonal fruit like mango which represented about 15% of the intake is introduced. If mango replaced other selenium containing food item it would not differ, but mango seemed to be eaten instead of sugar canes which did not contain selenium. You would therefore expect a rise in selenium intake.

In Donovan's study the children in preharvest season (November) had the lowest median selenium intake compared to harvest (March-April) and postharvest season (June-July) (36). This could indicate that intake found in this study represents a low intake during the year. Another study from Malawi dividing the year in harvest (April-July), postharvest (August-November) and preharvest (December-March) found another conclusion. The intake of macronutrients, zinc and vitamin A and C were higher from April to November than December to March (45). This division corresponds better to the findings from Mangochi, and indicates that the December-March intake of selenium and sulphur amino acids could be lower than found in this study.

As most of the participants harvested in April 2006 and were heavily dependant on locally produced food, the likelihood for shortages of food increased the closer they got to next harvest in April 2007. If harvesting the crop in April and the median duration was 7 months for the crop to last, it will end in November. The main crop was maize which represented 23-26% of the selenium intake. It could be that the increase of mango compensated for the decrease of maize and the selenium intake equalised. Fish were the main source of selenium intake and availability are probably not influenced by seasons. In Donovan's study the proportion of selenium from fish increased while proportion of selenium from cereal products decreased from June to November. Legumes' contribution of selenium intake decreased from March to July and stayed low (36). This could indicate that storage of cereals last longer than legumes and fish get increasingly important of selenium intake as people get less staple food. This should count for sulphur amino acids as well as the main contributors a similar.

For cysteine and methionine, maize represented 34-51% of the overall intake. Still no increase or decrease was seen. Maybe this was because many interviews in November or that the decrease had not shown yet. A general feeling after unstructured observation suggested that number of participants with low food intake increased during the study period. The statistical calculations did not find a seasonal difference of intake on population basis.

As seen from Figure 3.10, TB patients have a wider distribution of which month they are harvesting. This could reflect the fact that they are sick and have problems of harvesting the entire crop. To manage they harvest over a longer time period. When the participants answered they indicated one month each and not a period. The question could still be interpreted by the participant as when starting to harvest or ending his/her harvest. This supports the indication that TB patients need more time to do their harvest. It did not seem that had any influence on the size of harvest as the duration of how long time the harvest lasted did not differ between the two groups.

4.2.5 Nutritional factors

The intake that is compared in this study is the selenium, cysteine and methionine eaten. Other factors like bioavailability, digestion, absorption, composition of diet and intake of antinutrients may influence the utilisation of the mineral or amino acid. The composition of diet in the two groups was very much alike. Selenium is absorbed in the small intestines and is not under homeostatic control. Selenium is normally well absorbed in humans with over 80% of selenite and over 90% of selenmethionine and selenate (4). Bioavailability of selenium in human food varies from food item to food item. Animal studies suggest that selenium from wheat, beef kidney and Brazil nuts are utilized well. Bioavailability from fish and meat may be as low as 20-50% in birds and mammals (42). A study looking at the effect of organic and inorganic intake of selenium in animals found that rats had higher levels of selenium in all tissue after feeding on selenmethionine than selenite. No difference in GSHPx activity was found (51). This could mean that the participants from Mangochi had a low use of intake as most of their selenium came from fish.

A main antinutrient to selenium is iodine. Selenium interacts with iodine balancing the hormone production (52). The controls and TB patients had more or less similar intake of the mineral. The median intakes in the two groups were 236 and 253 µg/day which is well above

the RNI of 150 µg/day for adults (42). The basis of these results is South African values, and the level of iodine varies a lot geographically. Mercury exposure has shown to lower the activity of selenoenzymes and seclusion of selenium. It is also known to bind complexes with sulphur (53). Other minerals may have an influence but an animal study found no effects of copper, cadmium, iron, manganese or molybdenum in selenium absorption (54).

The groups also have a similar intake concerning cassava which may contain cyanogens. In detoxification, sulphur amino acids are used in the synthesis of rodanese enzyme. This may represent an extra demand of the sulphur amino acid stores. Processing of the cassava lead to a reduction of cyanogens (55). About ¼ of the participants ate cassava either raw or cooked. This shows probably that the intake is not great overall but could be for the ones eating unprocessed bitter cassava now and then.

Other anti nutrients to sulphur amino acids that occur naturally are trypsin inhibitors, tannins, phytates and gossypol. Antinutrients may also be formed by food processing like heat treatment or addition of processing agents. This may lead to formation of oxidized forms of methionine and cysteine such as methionine sulfoxide, methionine sulphone and cysteic acid (56). A study done in animals analysing the nutritional level of sulphur amino acids in raw and sterilised milk and fish protein found difference in bio availability. Cod protein and casein subjected to processing had lower digestibility, assimilate ability and growth yield of methionine, cysteine and taurine than raw protein. The study also found lower serum levels of biological active sulphur amino acids after eating sterilised proteins than raw (57).

Another consideration may be that amino acids are normally absorbed in the small intestines after a complex protein digestion and are transported with the bloodstream to the liver. Surplus of amino acids are stripped for the NH2 group and transformed into glucose (31). As both components are absorbed in the small intestines absorption would probably be negatively affected by diarrhoea (58).

4.2.6 Content in food items

4.2.6.1 Sulphur amino acids

7 local food items were analysed for the content of sulphur amino acids. The results were compared to the food composition tables FF3 and KFB. The mean difference in percentage from the highest value between local values and food composition values were 23% for methionine and 11% for cysteine. This indicates low geographical variation and the validity of using food composition values in calculation of intake for the food items where local values were not measured. Fish and gruel values were not comparable. The percentage difference from the highest value between local *Usipa* value and food composition fish value were 71% and 59% for cysteine and methionine. *Usipa* fish was the smallest fish and was eaten with intestines. This could be the reason for high sulphur amino acid values or it could be type specific. *Usipa* had in addition high protein content and a proportionally high sulphur amino acid content of total protein. For other food items the mean percentage of differences from the highest value between the values in the two composition tables was 20 and 21% for cysteine and methionine respectively.

Table 4.3: Comparison of sulphur amino acid values

		Mangochi (mg/g)	FF3/KFB (mg/g)
Stiff maize porridge, white	Methionine	0,6	0,43
	Cysteine	0,5	0,55
Maize flour, brown	Methionine	1,5	1,7
	Cysteine	1,9	1,46
Usipa fish	Methionine	18	7,4
	Cysteine	7,9	2,2
Kidney beans	Methionine	2,4	3,34
	Cysteine	2,2	2,24
Gruel/Beer	Methionine	0,1	0,01
	Cysteine	0,1	0,03

4.2.6.2 Selenium

The selenium content in food items may vary a lot geographically due to content of selenium in soil and the bioavailability predicted by soil texture, pH in soil, chemical form of selenium, organic matter content and content of competing ions (4). Looking at the results in Table 3.6 in this study such a variation is seen. Borehole water and tomatoes collected from areas with different soil show different values. Variances were also seen between white and red onion, between white maize flour from fertilized garden and not fertilized garden and between

different subtypes of ground nuts. If these differences are due to fertilizing, type, subtype, geographical variances or other factors are hard to tell but it gives an understanding of the variability of selenium content. Even if only one analysis was done per food item, the composite samples were maid of many food items. A high number will assumingly represent better the average. Most of cereal, legumes, nuts and some vegetables samples are made of more than 20 items.

Small fish like *Usipa* with intestines which was dried has much higher selenium content than fresh *Chambo* which was a larger species. This could be due to the water content but also that fish meat had lower levels of selenium than fish meat and intestines together. Fish like *Utaka* and *Kambusi* were as well small fish and indicates that high content in *Usipa* is related to type and not only size. Cereal had less than 1/10 of the content of fish with rice lower than sorghum and maize. Vegetables and fruit had generally a low content with the exception of pumpkin leaves and turnips that had high level of selenium. Mango had 10 times higher level than paw paw and 3 times as banana. The variation continued between legumes with high content in hyacinth bean, cow peas and pigeon peas with lower in kidney beans. Spices and herbs had a high content of selenium seen in curry, pepper, tea leaves and neem.

The results found in Mangochi may be compared to one study done in neighbouring Zomba district in Malawi. In the study from Zomba a composite sample of 1-10 sub samples were collected and analysed for selenium with instrumental neutron activation analysis (35). Unrefined maize flour analysis from Mangochi shows the double of content than in Zomba. The extraction rate of white maize flour is not known from the samples from Mangochi and is therefore not compared. In the study done in Zomba the content of selenium in unfermented, fermented, refined and cooked maize flour was measured. It found only little loss of selenium after processing (35). A study done on wheat grain had a similar conclusion. Selenium was more evenly distributed than other minerals and no losses were found during processing (47). In this study selenium levels are slightly higher in white maize flour than brown maize flour and it is supported with the results from white stiff maize porridge with higher selenium values than brown stiff maize porridge.

8/12 of the compared food items in Table 4.4 had higher selenium content in Mangochi than in Zomba district. This is probably not a result of choosing different analysing method as discussed before, because the differences are quite large. There may be regional differences

but the comparison may question the reliability of the high selenium content in mango, pigeon pea and in hyacinth bean, and the samples may have gotten polluted before analyses.

Table 4.4: Comparing selenium values from Mangochi and Zomba district.

	Mangochi district	Zomba district
Maize flour	4,9	2,5 +/- 0,8
Rice	1,0	2,4 +/- 1,0
Sorghum	8,4	12,9 +/- 3,9
Pumpkin leaf	7,0	nd
Turnip leaf	11,7	nd
Banana	0,9	0,5 +/- 0,3
Mango	2,7	0,6 +/- 0,1
Hyacinth bean	28,6	1,3 +/- 0,4
Kidney bean	2,2	0,7 +/- 0,3
Pigeon pea	15,5	5,6 +/- 1,5
Chambo	6,8	26,2 +/- 4,9
Matemba, fresh		11,1 +/- 3,8
Usipa, dried	84,2	73,2 +/- 1,7

µg/100g edible portion (+/-SD) (page 4-5)(35)
nd – not detectable

The content in *Usipa* and banana is quite similar. Pumpkin and Turnip leaves from Zomba were boiled in 20 minutes before analysing contrary to raw leaves from Mangochi. From Zomba 4 and 6 leaves were analysed compared to 5 and 10 in Mangochi. 8 bananas and 10 mangoes were analysed in Zomba compared to 3 and 5 in Mangochi. The *Chambo* analysed from Zomba were dried compared to the one from Mangochi which was fresh and had more similar moisture as the *Matemba* from Zomba. From Mangochi only 1 *Chambo* was the base for the value compared to 7 for the Zomba value. For cereal and legumes > 20 items made the composite sample analysed in Mangochi compared to in Zomba where 3-10 items were collected.

The content of selenium in plants may vary and it is not obvious that a plant in seleniferous area has highest content. Even so compared to values from around the world the values from Mangochi could indicate the soil content of selenium to be adequate (4). The content of selenium measured from borehole water could tell us something about the general content of selenium in the area. Compared to other analyses done around the world the freshwater from Malawi (0,2-0,8µg/L) had 10 times higher values than world freshwater and seawater but 100-1000 times lower than seleniferous water (4). 1/3 of the participants reported burning of the vegetation and 15% reported use of sulphur containing fertilizer, practises that could cause depletion of selenium in soil and low bioavailability of selenium to plants respectively (4).

4.2.7 Body mass index

BMI in TB sputum positive patients significantly differed from the BMI in controls. A finding also found in earlier studies (17). The standard deviations of the two means are larger than the mean difference. This means that the average of the distance each value has to the mean are bigger than the difference between the groups. This indicates that the distribution of BMI in the 2 groups is rather high and overlapping. Analysing for the different genders showed no significant difference between the two means for women. This is probably because of a large standard deviation of the women's means in the two groups due to several outliers in a limited number of cases. The outliers are included in the analyses as the values are within a normal range and no errors detected. Low BMI has been associated with severity of lung disease, mortality and selenium deficiency (14-16).There was no correlation between BMI and intake of selenium, methionine or cysteine in this study. As earlier studies investigating intake of selenium or sulphur amino acid are not found, this is an interesting finding. This result has to be considered on its basis where only one day intake is measured.

An assumption is that TB patients have worse nutritional status the longer they have had symptoms of tuberculosis. Such an indication is not found in this study. There was not found a negative or positive correlation between BMI and duration of symptoms. This may indicate that individual variation have a stronger influence than thought. These results are influenced by different period of recovery for the patients and difficult to interpret.

A lower BMI in TB patients may be discussed in light of composition of diet and amount of macronutrient intake. In this study it was found a significant difference between the groups in consumption of stiff maize porridge which consist mainly of carbohydrates. Stiff maize porridge was the food item eaten in largest amount and this corresponds with that carbohydrates are the main energy source in developing countries (31). TB patients had a higher intake of fat than controls though not significantly. Protein intake was not measured. On this basis controls would probably have a higher energy intake than TB patients. An explanation to lower BMI is that TB influences the distribution of nutrients in the body and that the body uses more macro and micronutrients to fight the disease. It is known that during an infection the body looses nitrogen (31). The major components of the body weight are fat and fat free mass and a proportion varies on individual, gender and ethnic basis. The fat free mass consist of water 72%, protein 19%, mineral 8%, glycogen 1-2% and about 50% of the water are intracellular. When loosing weight both fat and fat free mass is lost but the

proportion of fat free mass is dependant on fat storage (59). In a population with low fat storage the loss of fat free mass would assumingly be greater if getting sick. The TB patients have as well probably gone through a period of illness with lower appetite than the recording day in this study.

The participants measured close to 60 days of treatment did not have a higher BMI than the ones measured just after 2 weeks. As the patients are not followed this does not contradict earlier findings showing a significant increase of weight after 4 weeks on treatment (17). The graph of weight to duration of treatment indicates still that there might be a separation of TB patients of who have higher weight. A suggestion would be that TB patients co-infected with HIV have a lower BMI than TB patients. This is supported by the findings in one study from Burundi showing that HIV positive patients with pulmonary TB had a significantly lower BMI, fat free mass and body fat than HIV negative patients with pulmonary TB (60).

4.2.8 Risk factors

Earlier studies have shown that TB is associated with poverty within a community. A study from Liverpool, England found a significant correlation between poverty indices and rate of TB within a community (37). This study did not find poverty associated with the TB patient group compared to controls. To the contrary the study found good housing conditions with wooden or cement floor as a significantly high odds ratio of 11,8 of being TB patient in multivariate analysis. There was a wide confidence interval indicating that the risk may not be high. This finding is supported by higher odds ratio of having TB for fair socioeconomic status compared to poor, though not significant. Socio economic indices like assets and animals may vary on short time depending on need of cash or an occasionally high salary, gifts etc. Housing condition like type of flooring reflects assumingly more long term socio economic status or background if not an earlier intervention helped the poorest to build houses with cement floor. Based on earlier findings poverty is a general problem in Malawi with over half of the population under the national poverty line (5). This could mean that the major poverty in the general society overshadows minor differences between TB patients and controls. Another factor not to forget is HIV which does not discriminate much between rich and poor. 1/5 of people in Mangochi have HIV and prevalence increases with socioeconomic status (1). A high prevalence rate would have an impact on the whole community. As known HIV positive are more prone to get TB infection and many of the TB patients have HIV (11).

This could have the influence that TB patients come from a wider socioeconomic population than before.

Higher education showed as a strong and significant protective factor for having TB in multivariate analysis, a finding that at first contradicts the risk of having good housing conditions. It is not enough to know how to read as the preventive effect starts after having 8 years of education. This could mean low access to health education or that the material has not been fitted to people with low educational level. Concerning the contradiction with wealth, it might be that education is not associated with higher wealth. A reason could be a selection of TB patients in the two hospitals. Central hospitals in the two big cities were about 4 hours of drive away and known to be better. If the wealthiest TB patients travelled away for treatment this result may make sense. The contradicting result could also be because of a small sample or confounding factors not counted for.

No socioeconomic factors had a significant protective effect for having a one day deficient selenium or cysteine intake in multivariate analysis. This may be because the intake is based on only one 24 hour recall and not representative for the true individual intake. The result has to be considered also in the perspective that the information is collected in a matched case-control study and there might be connections between the data not counted for when restructuring groups.
The results found an increasing age as a significant risk factor for having a one day deficient intake. This result is supported as it is for both cysteine intake and selenium intake. The odds ratio is small and just slightly higher than 1 but strong and had a narrow 95% confidence interval. Though not significant, higher socio economic status based on assets and flooring was a risk for having a one day deficient selenium intake, but a protective factor for having a one day deficient cysteine intake.

5 Conclusions

TB sputum positive patients did not have a significantly lower intake of selenium and sulphur amino acids than healthy adults from Mangochi district. Participants in this study had a low intake of selenium and an adequate intake of sulphur amino acids. The control group had a significantly higher intake of stiff maize porridge but a significantly lower diversity in the diet. The proportion of selenium and sulphur amino acid intake from different food types was similar in the two groups. Fish and maize were the main contributors for both selenium and sulphur amino acids. The TB sputum positive patients had a significant lower BMI than the controls. This study indicates that the deficiencies of selenium and thiols found in earlier studies may not entirely be due to a low intake of selenium and sulphur amino acids.

5.1 Recommendations

5.1.1 TB patients

Earlier studies have shown wasting in TB patients associated with increased mortality and deficiency in serum selenium level. Serum deficiency in thiols is also documented. This study indicates that this is due to increased body consumption. Deficiencies in serum could probably be compensated by an increased intake. Socioeconomic characteristics found in this study indicate that households probably do not have means to care for an increased intake. Recommendations from this study would therefore be to support TB patients with selected food rich in selenium and sulphur amino acids during treatment. Further studies are recommended to see if an increased intake of selenium and sulphur amino acids has implications on recovery and mortality of TB sputum positive patients.

5.1.2 Community

The authorities and donors may assess the need of adding selenium to fertilizers. Finding ways of dealing with bush in the fields and not burning the vegetation may be a part of the strategy of keeping selenium content in soil. Health educators could promote intake of food items that would be good for both selenium and sulphur amino acid intake like small fish, mango, hyacinth beans, peas, turnips and pumpkin leaves. Milk from goat and cow and poultry should be recommended if affordable.

Reference List

(1) Malawi Demographic and Health Survey 2004. Zomba: National Statistical Office and ORC Macro; 2005.

(2) Pearson Education. U.S. Department of State Background Note on Malawi. http://www infoplease com/country/profiles/malawi html 2007

(3) CIA-The world factbook-Malwi. https://www cia gov/cia/publications/factbook/geos/mi html 2007

(4) Fordyce F. Selenium Deficiency and Toxicity in the Environment. In: Selinus O, Alloway BJ, Centeno JA, Finkelman RB, Fuge R, Lindh U, et al., editors. Essentials of Medical Geology - Impacts of the Natural Environment on Public Health.China: Elsevier Academic Press; 2005. p. 373-416.

(5) Integrated Household Survey 2004 - 2005. Zomba: National Statistical Office; 2005.

(6) Department of Planning MoH. A joint programme of work for A Health Sector Wide Approach, 2004-2010. Lilongwe: Republic of Malawi; 2004.

(7) Kulmala T, Vaahtera M, Ndekha M, Cullinan T, Salin ML, Koivisto AM, et al. Socio-economic support for good health in rural Malawi. East Afr Med J 2000 Mar;77(3):168-71.

(8) World Gazetteer. http://world-gazetteer com/wg php?x=&men=gadm&lng=en&dat=32&geo=-150&srt=npan&col=aohdq 2006

(9) Food and Agriculture Organization, World Food Programme. FAO/WFP CROP AND FOOD SUPPLY ASSESSMENT MISSION TO MALAWI. Rome; 2003 Jun 9.

(10) World Health Organization. Tuberculosis - the global burden. internet 2005Available from: URL: http://www.who.int/tb/publications/tb_global_facts_sep05_en.pdf

(11) Harries AD, Hargreaves NJ, Gausi F, Kwanjana JH, Salaniponi FM. High early death rate in tuberculosis patients in Malawi. Int J Tuberc Lung Dis 2001 Nov;5(11):1000-5.

(12) World Health Organization. TB epidemiological profile. internet 2005 June 1Available from: URL: http://www.who.int/GlobalAtlas/predefinedReports/TB/PDF_Files/mwi_2003_brief.pdf

(13) Schwenk A, Macallan DC. Tuberculosis, malnutrition and wasting. Curr Opin Clin Nutr Metab Care 2000 Jul;3(4):285-91.

(14) van Lettow M, Harries AD, Kumwenda JJ, Zijlstra EE, Clark TD, Taha TE, et al. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. BMC Infect Dis 2004 Dec 21;4(1):61.

(15) van Lettow M, Kumwenda JJ, Harries AD, Whalen CC, Taha TE, Kumwenda N, et al. Malnutrition and the severity of lung disease in adults with pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis 2004 Feb;8(2):211-7.

(16) Zachariah R, Spielmann MP, Harries AD, Salaniponi FM. Moderate to severe malnutrition in patients with tuberculosis is a risk factor associated with early death. Trans R Soc Trop Med Hyg 2002 May;96(3):291-4.

(17) van Lettow M, Fawzi WW, Semba RD. Triple trouble: the role of malnutrition in tuberculosis and human immunodeficiency virus co-infection. Nutr Rev 2003 Mar;61(3):81-90.

(18) Kassu A, Yabutani T, Mahmud ZH, Mohammad A, Nguyen N, Huong BT, et al. Alterations in serum levels of trace elements in tuberculosis and HIV infections. Eur J Clin Nutr 2005 Dec 7.

(19) Madebo T, Lindtjorn B, Aukrust P, Berge RK. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. Am J Clin Nutr 2003 Jul;78(1):117-22.

(20) Range N, Changalucha J, Krarup H, Magnussen P, Andersen AB, Friis H. The effect of multi-vitamin/mineral supplementation on mortality during treatment of pulmonary tuberculosis: a randomised two-by-two factorial trial in Mwanza, Tanzania. Br J Nutr 2006 Apr;95(4):762-70.

(21) Levinson W. Medical Microbiology & Immunology: Examination & Board Review. eighth ed. New York: Lange Medical Books/McGraw-Hill; 2004.

(22) Beckett GJ, Arthur JR, Miller SM, McKenzie RC. Selenium. In: Huges DA, Darlington LG, Benedich A, editors. Diet and Human Immune Function. 1 ed. New Jersey: Humana Press Inc.; 2004. p. 217-40.

(23) Grimble RF. Sulphur Amino Acids, Glutathione and Immune Function. In: Calder PC, Field CJ, Gill HS, editors. Nutrition and Immune Function. 1 ed. New York: CABI Publishing; 2002. p. 133-50.

(24) Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. Public Health Nutrition 2001;4(2B(Special)):593-9.

(25) Tapiero H, Townsend DM, Tew KD. The antioxidant role of selenium and seleno-compounds. Biomed Pharmacother 2003 May;57(3-4):134-44.

(26) Arthur JR, McKenzie RC, Beckett GJ. Selenium in the immune system 11th International Symposium on Trace Elements in Man and Animals. June 2-5, 2002, Berkeley, California, USA. Journal of Nutrition 2003;133(5, Supplement 1):1457S-9S.

(27) Duzova H, Asma D, Emre MH. Neutrophil superoxide anion production, and CAT and GSSGR activity in patients with tuberculosis. New Microbiol 2003 Jul;26(3):289-98.

(28) Green RM, Seth A, Connell ND. A peptide permease mutant of Mycobacterium bovis BCG resistant to the toxic peptides glutathione and S-nitrosoglutathione. Infect Immun 2000 Feb;68(2):429-36.

(29) Combs GF. Geological Impacts on Nutrition. In: Selinus O, Alloway BJ, Centeno JA, Finkelman RB, Fuge R, Lindh U, et al., editors. Essentials of Medical Geology - Impacts of the Natural Environment on Public Health.China: Elsevier Academic Press; 2005. p. 161-77.

(30) Parcell S. Sulfur in human nutrition and applications in medicine. Altern Med Rev 2002 Feb;7(1):22-44.

(31) Latham MC. Macronutrients: carbohydrates, fats and proteins. Human nutrition in the developing world.Rome: Food and Agriculture Organization; 1997. p. 255-70.

(32) Benemariya H, Robberecht H, Deelstra H. Daily dietary intake of copper, zinc and selenium by different population groups in Burundi, Africa. Sci Total Environ 1993 Aug 15;136(1-2):49-76.

(33) Labadarios D, Steyn NP, Maunder E, MacIntryre U, Gericke G, Swart R, et al. The National Food Consumption Survey (NFCS): South Africa, 1999. Public Health Nutr 2005 Aug;8(5):533-43.

(34) Maleta K, Kuittinen J, Duggan MB, Briend A, Manary M, Wales J, et al. Supplementary feeding of underweight, stunted Malawian children with a ready-to-use food. J Pediatr Gastroenterol Nutr 2004 Feb;38(2):152-8.

(35) Donovan U, Gibson RS, Ferguson EL, Ounpuu S, Heywood P. The selenium content of staple foods from Malawi and Papua New Guinea. Journal of Food Composition and Analysis 1991;4(4):329-36.

(36) Donovan UM, Gibson RS, Ferguson EL, Ounpuu S, Heywood P. Selenium intakes of children from Malawi and Papua New Guinea consuming plant-based diets. J Trace Elem Electrolytes Health Dis 1992 Mar;6(1):39-43.

(37) Spence DP, Hotchkiss J, Williams CS, Davies PD. Tuberculosis and poverty. BMJ 1993 Sep 25;307(6907):759-61.

(38) Gibson RS, Ferguson EL. An Interactive 24-hour Recall for Assessing the Adequacy of Iron and Zinc Intakes in Developing Countries. Washington D.C.: ILSI Press; 1999.

(39) International Organization for Standardization. ISO 13903:2005 Animal feeding stuff -- Determination of amino acids content. 1, 1-17. 2-6-2005. International Organization for Standardization.
Ref Type: Serial (Book,Monograph)

(40) THE COMMISSION OF EUROPEAN COMMUNITIES. Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feedingstuff and amending Directive 71/393/EEC (Text with EEA relevance). Official Journal of the European Communities 1998 Sep 19;L 257:14-28.

(41) World Health Organization, Food and Agriculture Organization, UNU. Energy and proteins requirements. Switzerland; 1985.

(42) World Health Organization, Food and Agriculture Organization. Vitamin and mineral requirements in human nutrition. China; 2004. Report No.: Second.

(43) Takyi EE, Amankwa P. Dietary selenium and copper intake by resident undergraduate students of the University of Ghana. East Afr Med J 2004 Jan;81(1):34-9.

(44) Ferguson EL, Gadowsky SL, Huddle JM, Cullinan TR, Lehrfeld J, Gibson RS. An interactive 24-h recall technique for assessing the adequacy of trace mineral intakes of rural Malawian women; its advantages and limitations. Eur J Clin Nutr 1995 Aug;49(8):565-78.

(45) Nyambose J, Koski KG, Tucker KL. High intra/interindividual variance ratios for energy and nutrient intakes of pregnant women in rural Malawi show that many days are required to estimate usual intake. J Nutr 2002 Jun;132(6):1313-8.

(46) Higgs DJ, Morris VC, Levander OA. Effect of cooking on selenium content of foods. J Agric Food Chem 1972 May;20(3):678-80.

(47) Lyons GH, Genc Y, Stangoulis JC, Palmer LT, Graham RD. Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. Biol Trace Elem Res 2005 Feb;103(2):155-68.

(48) Hurrell RF, Finot PA. Food processing and storage as a determinant of protein and amino acid availability. Experientia Suppl 1983;44:135-56.

(49) Abdulla M, Behbehani A, Dashti H. Dietary intake and bioavailability of trace elements. Biol Trace Elem Res 1989 Jul;21:173-8.

(50) Food and Agriculture Organization. Food energy - methods of analysis and conversion factors. 2003.

(51) Whanger PD, Butler JA. Effects of various dietary levels of selenium as selenite or selenomethionine on tissue selenium levels and glutathione peroxidase activity in rats. J Nutr 1988 Jul;118(7):846-52.

(52) Foster LH, Sumar S. Selenium in health and disease: a review. Crit Rev Food Sci Nutr 1997 Apr;37(3):211-28.

(53) Raymond LJ, Ralston NVC. Mercury: selenium interactions and health implications. Seychelles Medical and Dental Journal 2004 Nov;7(1):72-7.

(54) bdel Rahim AG, Arthur JR, Mills CF. Effects of dietary copper, cadmium, iron, molybdenum and manganese on selenium utilization by the rat. J Nutr 1986 Mar;116(3):403-11.

(55) Ngudi DD, Kuo YH, Lambein F. Cassava cyanogens and free amino acids in raw and cooked leaves. Food Chem Toxicol 2003 Aug;41(8):1193-7.

(56) Gilani GS, Cockell KA, Sepehr E. Effects of antinutritional factors on protein digestibility and amino acid availability in foods. J AOAC Int 2005 May;88(3):967-87.

(57) Lipka E, Ganowiak Z. [Nutritional value of protein subjected to technologic processing. Changes in content of biologically active amino acids--methionine, cysteine and taurine under the influence of sterilization]. Rocz Panstw Zakl Hig 1993;44(2-3):151-8.

(58) Jacob SW, Francone CA, Lossow WJ. Fordøyelsessystemet. In: Dietrichs E, Hurlen P, editors. Anatomi og Fysiologi. Second ed. Oslo: Universitetsforlaget; 1985. p. 407-70.

(59) World Health Organization. Physical status: The use and interpretation of anthropometry. Geneva: Report of a WHO Expert Committee; 1995.

(60) Niyongabo T, Henzel D, Idi M, Nimubona S, Gikoro E, Melchior JC, et al. Tuberculosis, human immunodeficiency virus infection, and malnutrition in Burundi. Nutrition 1999 Apr;15(4):289-93.

Appendix 1: Recording form for 24 hour recall

Recording form for 24 hour recall				
Interview date: Day food eaten: Weight: Height: TB sputum positive□ Control□			Village: Subject id: Age: Sex:	
Time	Place eaten	Food or drink, description and cooking method	Amount eaten	Weight equivalent (g)

Was food intake unusual? Yes □ No □
Kodi chakudya chomwe munadya ndichachilendo?
Chakulya chiwalirecho chaliji chachilendo?

If yes, how was it unusual?
Ngati chili chachilendo ndi chachilendo bwanji?
Naga elo, chaliji chachilendo chamtuli?

Were you sick yesterday? Yes □ No □
Kodi munadwala dzulo?
Waliji nkulwala liso?

If yes, did sickness affect the appetite? Yes □ No □
Ngati munadwala, chilakolako cha chakudya chinaonongeka?
Naga elo, kulwalako kwatendekasisye kuti akasachiona kunong'a chakulyacho?

If yes, how?
Ngati inde, chinaonongeka bwanji?
Naga elo, chamtuli?

Was it a feast day? Yes □ No □
Kodi linali tsiku la phwando?
Lyaji lisiku lya chisangalalo?

Did you take any tablets or herbs yesterday? Yes □ No □
Munamwa mankhwala amapilisi kapena achikuda ena ali wonse dzulo?
Wamwere mtera wamapilisi kapena wachikuda wine wuli wose liso?

If yes, which kind of tablets or herbs?
Ngati inde, munamwa mankhwala amapilisi kapena achikuda anji?
Naga elo, galiji mapilisi kapena ntelachi wachikuda?

Where did you buy them?
Munagula kuti mankhwalawo?
Wagasumile kwapi mapilisigo?

EDUCATION/ RELIGION/ WORK

Do you know how to read? Yes □ No □
Kodi mumadziwa kulemba?
Akusakombolaga kuwaranga?

How many years at school have you completed?
Kodi munakhala zaka zingati ku sukulu?
Kusukulu wasomile yaka iliungwa?

Which religious group do you belong to? Christian □
Ndinu ampingo wanji? Muslim □

Wawo dini jawo chi? Other □

What is your occupation? Subsistent farmer □
Mumagwira ntchito yanji? Commercial farmer □
Akusakamula masengo gachi? Subsistent fisherman □
Commercial fisherman □
Formal employment □
Other □

If farmer, do you use fertilizer in your field? Yes □ No □
Ngati muli Mlimi, mumagwiritsa ntchito feteleza m'munda mwanu?
Naga mlimi, akusatagagafeteleza mum'gunda mwawo?

If yes, what kind? 23/21/04S □
Ngati inde, feteleza wake wamtundu wanji? Urea □
Naga elo, mtunduchi wafetelezajo? CAN □
D compound □
Other □

Did you burn your field to clean it? Yes □ No □
Kodi munaotcha m'munda mwanu pososa?
Akusajocha m'gunda wawo pakusaka kulangusya?

When do you usually harvest?
Ndi nthawi yanji yomwe mumakonda kukolola?
Mnopemnope akusagoolaga mwezichi?

When does your crop end?
Ndi mwezi wanji womwe zokolola zanu zimatha?
Yagoola yawo ikusamalaga Mwesichi?

POSESSIONS

Does your household have a blanket? Yes □ No □
Kodi pakhomo panu pali bulangete?
Ana mnyumba mwawo wana libulangeti?

Does your household have a radio? Yes □ No □
Kodi pakhomo panu pali wailesi?
Ana mnyumba mwawo wana wailesi?

Does your household have a mattress? Yes □ No □
Kodi pakhomo panu pali matress?
Ana mnyumba mwawo wana matilesi?

Does your household have a bicycle? Yes □ No □
Kodi pakhomo panu pali njinga yopalasa?
Ana mnyumba mwawo wana Njinga?

Does your household have a TV? Yes □ No □
Kodi pakhomo panu pali television kapena kanema?
Ana mnyumba mwawo wana TV?

Does your household have a boat for fishing? Yes □ No □
Kodi pakhomo panu pali bwato?
Ana mnyumba mwawo wana liboti kapena wato wakulajila somba?

Do you have shoes? Yes □ No □
Muli ndi nsapato?
Akwete sapato?

Does your family own land? Yes □ No □
Kodi banja lanu lili ndi malo?
Ana liwasa lyawo lyana malo gawogawope?

Does your household have domestic animals? Yes □ No □
Kodi banja lanu lili ndi ziweto?
Ana nyumba mwawo wana ilango?

If cows, how many?	
If goats, how many?	
If chicken, how many?	
If guiniea foul, how many?	

HOUSING CONDITIONS

Where do you get your drinking water from?
Kodi madzi akumwa mumatunga kuti?
Mesi gakumwa akusatecheraga kwapi?

Tap or borhole □
Unprotected well/lake □

How long does it take you to get to the watersource?
Mumatenga nthawi yaitali bwanji kukafika kotunga madzi?
Akusajenda ndawi jelewu uli kuti akaiché kwakusatecheraga mesiko?

< 15 min □
30 min □
45 min □
> 1 hour □
Don't know □

What kind of flooring is there in your house?
Kodi pansi pa chimbudzi munazila ndi chani?
Pasi panyumba jawo wasyasyajile nichi?

Sand/dung or earth □
Wood or cement □

What kind of toilet do you use?
Ndi mtundu wanji wa chimbudzi chomwe mumagwiritsa ntchito?
Akusaka mulisya masengo chimbuzi chachi?

Traditional pit latrine □
Traditional pit latrine with san plat □
Flush toilet □
None □

Appendix 2: Invitation to TB patients – English

Invitation to participate in the study:

Project title: Food intake of selenium and sulphur amino acids in tuberculosis patients and healthy adults in Malawi.

Tuberculosis is a serious disease that affects an increasing number of people each year in Malawi. Tuberculosis is causing weight loss and poor appetite. Nutritional food is important in order to gain weight and recover from the disease. The trace element selenium and the sulphur amino acids are essential for the immune system and therefore also the healing process in TB patients.

This study aims to investigate the intake of selenium and sulphur amino acids in tuberculosis patients on treatment and properly matched healthy control group. This study will give information on the dietary intakes of these two groups. This study will also give indications what kind of food that is rich in selenium and sulphur amino acids, and how much they are eaten by the population in this district. This information can be used as a basis for studies in the future where supplementary interventions may be suggested.

Information on the food intake will be collected through an interview where the participant will tell all that he/she had to eat and drink the day before. The researcher will also measure the participant's height and weight.

Who is invited to participate?
Patients with pulmonary tuberculosis who have started on their tuberculosis medication 2-8 weeks ago and appropriately matched healthy people are invited to participate. All participants should live in the Mangochi district.

Information to the participants:
You ought to know that your participation in this study is voluntarily and without binding or honorarium. If you agree to participate in this study; please sign in the attached consent form. Even if you agree now to participate in this study, you should know that you are free to withdraw yourself from the study at any time. Your decision will have no repercussions for you personally. The information you have given will then not be used.

The study is funded by University of Oslo (UIO). The researcher has no financial bindings to any interest groups or companies. This study has been approved by the Ethical Committee at UIO, Norway and by College of Medicine research and Ethics Committee in Malawi.

Your information will be treated confidentially in a manner that ensures security. It is not assumed any risk or discomfort for the volunteers participating in this study. The researcher does not have any legal responsibility to provide any medical service to the participants. If more information is needed please contact Dr Maleta at Mangochi District Hospital Tel 08 232 202 or Stephano Mwaliwa, Tel 09692799.

Information on the results

When this study is completed (May 2007), the researcher will send a report containing results to the relevant district health officer and other health institutions that are interested. The report will be available for the participants as well.

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International Health Section

Appendix 3: Invitation to TB patients – Chichewa

Muli kuitanidwa kutengapo mbali pa phunziro la:

Mutu wa Project: Kadyedwe ka selenium ndi sulphur amino acids mwa odwala chifuwa cha TB ndi a thanzi m'Malawi'

Chifuwa chachikulu cha TB ndi nthenda yoopsya kwambiri yomwe yakhudza anthu ochuluka chaka ndi chaka M'malawi. TB imapangitsa kuwonda komanso osakhala ndi chilakolako cha zakudya. Zakudya zopatsa thanzi ndi zofunika kwambiri kuti thupi linenepe komanso kuti munthu achire ku matendawa. Zakudya zing'onozing'ono za mineral selenium ndi za sulphur amino acids ndizofunika ku kayandetsedwe ka chitetezo cha thupi komanso zimathandizanso kuchiza matenda a chifuwa chachikulu cha TB.

Cholinga cha phunziroli ndikuzindikira za selenium ndi sulphur amino acids mwa munthu amene akudwala matenda a TB amene akulandira mankhwala ndi gulu la anthu a thanzi. Phunziroli lidzaonetsanso zizindikiro za mtundu wa chakudya chomwe chili ndi selenium ndi suphur amino acids wambiri komanso ndimene chiwerengero cha anthu chimadyera m'bomali. Ndondomekoyi ikhonza kugwiritsidwa ntchito ngati mfundo pamene maphunziro owonjezera pa phunziroli adzachitike mtsogolo muno.

Zambiri za katengedwe ka chakudyachi zidzachitika kudzera mmafunso amene otenga nawo mbali adzayankhe atadya chakudya ndi kumwa tsikuli lisanafike. Akafukufuku adzayezanso sikelo ndi msinkhu wamunthu.

Oyenera kutengapo mbali ndani?
Odwala TB onse amene ayamba kulandira mankhwala masabata awiri mpakana asanu ndi limodzi apatawo ndi anthu ena onse osankhika amene ali athanzi. Onse otengapo mbali akhale kuti amakhala m'boma la Mangochi.

Uthenga kwa onse otenga mbali:
Dziwani kuti kutenga kwanu mbali mu phunziroli ndi kofuna nokha komanso opanda kukakamizidwa. Ngati mwagwirizana nazo kuti mutenga mbali mu phunzirili chonde siinani pa chi fomu chomwe alumikizacho. Dziwani kuti muli omasuka kusiya phunziroli nthawi ili yonse. Chiganizo chanu sichingakhale ndi vuto lili lonse kwa inu ngati munthu. Zitakhala choncho ndiye kuti zomwe mwapelekazo sizingagwiritsidwe ntchito.

Phunziroli lapangidwe ndi chithandizo chochokera ku Univesite ya Oslo (UIO). Akafukufuku alibe chithandizo chilichonse cha ndalama kuchokera kwa anthu osangalatsidwa ndi phunziroli kapena makampani. Phunziroli ndilovomelezedwa ndi a Ethical Committee ku University ya UIO, Norway ndi a College of Medicine Research and Ethics Committee ku Malawi.

Maganizo anu adzasungidwa mwa chinsinsi komanso ndi chitetezo.Palibe vuto lina lili lonse losowetsa mtendere kwa wina ali yense amene wadzipeleka kutenga nawo mbali pa phunziroli. A kafukuku alibenso udindo wina uli wonse opeleka chithandizo cha mankhwala kwa anthu otenga nawo mbali. Ngati mungafune kudziwa zambiri mukhonza kupeza Dr. Maleta ku College of Medicine pa Mangochi District Hospital Tel 08 232 202 kapena Stephano Mwaliwa, Tel 09692799.

Uthenga wa zotsatira
Pamapeto pa phunziroli (May 2007), Ofufuza adzatumiza chikalata cha momwe zotsatila zakhalira kwa ma District Health Officers oyenerela ndi mabungwe a zaumoyo amene ali osangalatsidwa. Chikalatacho chidzakhalanso chopezeka kwa onse amene angatenge nawo mbali.

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Appendix 4: Invitation to TB patients – Chiyao

Akuwilangidwa kuti ajigale nawo mbali mu majiganyo ga:

Mtwe wa Pulojekiti:"Katupe ka yakulya ya selenium ni sulphur amino acids mwa wandu wakulwala TB ni wakwete thanzi m'Malawi".

Ulwele wa TB uli ulwele wakogoya m'nope waukamula wandu wajinji chaka chili chose m'Malawi. TB jikusamtendekasya mundu kuwa jwaganda nambo soni kusowa chilakolalako cha yakulya. Yakulya yakupeleka thanzi ili yakusosekwa kulimbikasya chilu soni ikusakamuchisya kulama ku chilwelechi. Jele selenium ji ni sulphur amino acids jili jakusosekwa ku chiteteyo cha m'chilu mwa mundu nambo soni ikusakamuchisya kuposya wakulwala TB.

Chakulinga cha gele majiganyoga ni chakuwungunya kajigale ka selenium ni sulphur amino acids mwa wandu wakulwala TB wakupochera mtera nigulu ja wandu wa thanzi m'chilu. Gele majiganyoga tigapeleche litala lya kajigale ka yakulya ya gele magulu gawiriga. Gele majiganyoga tigalosye soni mtundu wa yakulya ukwete selenium ni sulphur amino acids jwa m'jinji nambo soni kuti akulidwa mwejinji uli ni wandu wa m'boma ja Mangochi. Yitupate pelepayi komboleka kamulichisya masengo mpala mfundo sya majiganyo gane gakusogolo mpela gakonjechesya.
Utenga wakajigale ka yakulya tuchijigalidwa mkupitira kwausya mausyo wakujigala nawo mbali ni tachisala yosope yalire ni kumwa lisikulyo nkaniliyiche. Jwakaungunya tachalinga pasikelo soni kwalinga ulewu wawo.

Wani waliwakusosedwa kuti ajigale mbali?
Wosope wakulwa TB wakupochera mtera kutandira ijuma iwiri mpaka ijuma msano ni kamo yipiteyo, wandu wane wasagulidwe wathanzi akuwilanjidwa soni kuti ajigale nawo mbali. Wosope wakujigala nawo mbali awe wakutyochela m'boma ja Mangochi.

Utenga kwa wakujigala mbali
Amanyilire kuti kujigala kwawo mbali mu gele majiganyoga kuli kwakulipeleka, kwangachisya soni ngitwapa mbiya.
Naga ajitichisye kujigala nawo mbali pa gele majiganyoga, chonde asaine pachikalakala chalumbikenyocho. Atamose wawo akundile kujigala nawo mbali, amanyilire kuti wana ufulu wakugaleka majiganyoga ndawi jine jili jose. Nganisyo syawo pakutenda yeleyo pangali vuto kwa wawo mpela mundu. Naga ali atesile yaleyo nikuti utenga wosope waweleje alimkupeleka, ngasiukamulichisidwa masengo.

Majiganyoga gakukamuchisidwa ni wa University ja Oslo (UIO). Jwakaungunya jwangali chikamuchisyo cha mbiya kutyochela kwa gulu jawandu kapena makampani gakunonyelesedwa ni gale majigangyoga. Majigangyoga gali gakwitichika ni wa Ethical Committee ku UIO, Norway nambo soni wa College ja Medicine ni Ethics Committee ku Malawi.

Yitasalejeyo tiwe ya sili soni yakuteteyedwa. Pangali chakogoya chilichose kapena ngapata utendere kwa mundu jutalipeleche kujigala nawo mbali mu gele majigangyoga.
Jwakaungunya jwangali udindo uliwose wakupeleka chikamuchisyo cha mtera kwa wandu wakujigala nawo mbali. Naga akusaka kumanyinlila jejinji wasimane Dr Maleta ku College ja

Medicine ku chipatala chaboma ku Mangochi Tel 08 232 202 kapena Stephano Mwaliwa, Tel 09692799.

Utenga wa yakuyichisya
Patuchimalisya gele majiganyoga (May 2007), jwakaungunya tachitumisya m'ndandanda wa yakuyichisya kwa achakulungwakulungwa wa kulolela yipatala gagali gakunonyelesidwa. M'ndandandawo tuchiwa wakusimanikwa soni kwa wakujigala nawo mbali.

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Appendix 5: Consentform for TB patients – English

Declaration of consent for the study of

”Food intake of selenium and sulphur amino acids in tuberculosis patients and healthy adults in Malawi”.

I am hereby declaring that I have received information on the study “Food intake of selenium and sulphur amino acids in tuberculosis patients and healthy controls in Malawi.” I have been informed of the purpose of the above mentioned study, and that the information I will provide will be used for this study.

Besides, I have been informed that all the information I will provide will be treated with stringent confidentiality. Furthermore, I have been informed that the study has been approved by relevant authorities in Norway and Malawi.

I have also been notified that I can later withdraw from this study if I intend to do so in the future. I also know that the information pertaining to me can be used in similar study in the future.

Based on all this, I am hereby declaring the followings:

1. I agree voluntarily and without any reservation that I will participate in this study.

2. I agree that information I will provide can be used in similar study in the future.

Please cross on any item(s) to which you do not give your consent.

Participant signature Witness

Date and place Researcher signature, Frode Eick

Appendix 6: Consentform for TB patients – Chichewa

Declaration of consent for the study of

"Kadyedwe ka chakudya cha selenium ndi sulphur amino acids mwa odwala chifuwa cha TB ndi a thanzi m'Malawi".

Ndikunena pano kuti ndalandira ndondomeko ya phunziro "Kadyedwe ka chakudya cha selenium ndi sulphur amino acids mwa odwala chifuwa cha TB ndi a thanzi m'Malawi". Ndauzidwa za cholinga cha phunziro lomwe latchulidwa mmwambali, kuti zones zomwe ndingapeleke zigwiritsidwa ntchito mu phunziroli.

Komanso ndauzidwanso kuti ndondomeko yonse yomwe ndingapeleke idzatetezedwa kwambiri. Ndauzidwanso kuti phunziroli ndilovomelezedwa ndi maudindo akuluakulu ofunikira a dziko la Norway ndi Malawi.

Ndauzidwanso kuti ndikhonza kusiya phunziroli ngati ndingafune mtsogolo muno. Ndikudziwanso kuti chilichonse chomwe ndingapeleke chidzagwiritsidwa ntchito mu phunziro ngati lomweli mtsogolo muno.

Pambali pa mfundo zonsezi ndikuvomeleza izi:

3. Ndikuvomeleza kuti mwakufuna kwanga ndimosakakamizidwa ndidzatengako mbali mu mphunziroli.

4. Ndikuvomeleza kuti zonse zomwe ndapeleka zikhonza kudzagwiritsidwa ntchito pa mphunziro lofanana ndi lino mtsogolo.

Chonde dulani malo onse omwe simupelekapo maganizo anu.

Saini ya Wotengambali Mboni

Tsiku ndi malo Saini ya wakafukufuku, Frode Eick

Appendix 7: Consentform for TB patients – Chiyao

Kusala yakwitika kutenda majiganyo ga:

”Katupe ka yakulya ya selenium ni sulphur amino acids mwa wandu wakulwala TB ni wakwete thanzi m’Malawi”.

Une apano ngusala kuti mbochele utenga wa majiganyo ga ”Katupe ka yakulya ya selenium ni sulphur amino acids mwa wandu wakulwala TB ni wakwete thanzi m’Malawi”. Asalire ya chakulinga cha majiganyo ga galembedwe penanipa nikuti utenga utimbeleche tuchikamulichisidwa masengo mu gele majiganyoga.

Kupatula pele, asalire kuti utenga utimbeleche tuchiwa wa asili soni wakuteteyedwa. Nambo soni asalire kuti majiganyoga gajitichisidwe ni achakulungwakulungwa wa maudindo gawo wa ku Norway ni wa ku Malawi.

Asalire soni kuti mpaka ngomboleche kuleka gele majiganyoga naga ndili sachile yeleyo ku sogolo kuno.

Payosopeyi, une ngusala ayi:

1. Ngwitika mwakulipeleka soni mwangasunga nganisyo sine kuti tinjigale nawo mbali pa gele majiganyoga.

2. Ngwitika kuti utenga utimbeleche komboleka kamulichisya masengo mu majiganyo gakulandana ni gelega kusogolo kuno.

Chonde afute malo galigose gankanagasaka.

Saini ja wakusaina: Mboni:

Lisiku ni malo: Saini ja wakafukufuku, Frode Eick

Appendix 8: Invitation to controls - English

Invitation to participate in the study:

Project title: Food intake of selenium and sulphur amino acids in healthy adults in Malawi.

Mangochi district is located in the Great Rift Valley. In this area there may be a lot of igneous rocks with low concentration of selenium and sulphur in the soil. The trace element selenium and the sulphur amino acids are essential for the immune system and the health of people.

This study aims to investigate the intake of selenium and sulphur amino acids in healthy adults in Mangochi district. This study will give information on the dietary intake of this group. This study will also give indications what kind of food that is rich in selenium and sulphur amino acids, and how much they are eaten by the population in this district. This information can be used as a basis for studies in the future where supplementary interventions may be suggested.
Information on the food intake will be collected through an interview where the participant will tell all that he/she had to eat and drink the day before. The researcher will also measure the participant's height and weight.

Who is invited to participate?
Healthy people aged 15 to 60 are invited to participate. All participants should live in the Mangochi district.

Information to the participants:
You ought to know that your participation in this study is voluntarily and without binding or honorarium. If you agree to participate in this study; please sign in the attached consent form. Even if you agree now to participate in this study, you should know that you are free to withdraw yourself from the study at any time. Your decision will have no repercussions for you personally. The information you have given will then not be used.

The study is funded by University of Oslo (UIO). The researcher has no financial bindings to any interest groups or companies. This study has been approved by the Ethical Committee at UIO, Norway and by College of Medicine research and Ethics Committee in Malawi.

Your information will be treated confidentially in a manner that ensures security. It is not assumed any risk or discomfort for the volunteers participating in this study. The researcher does not have any legal responsibility to provide any medical service to the participants. If more information is needed please contact Dr Maleta at Mangochi District Hospital Tel 08 232 202 or Stephano Mwaliwa, Tel 09692799.

Information on the results
When this study is completed (May 2007), the researcher will send a report containing results to the relevant district health officer and other health institutions that are interested. The report will be available for the participants as well.

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International Health Section

Appendix 9: Invitation to controls – Chichewa

Muli kuitanidwa kutengapo mbali pa phunziro la:

Mutu wa Project: Kadyedwe ka chakudya cha selenium ndi sulphur amino acids mwa anthu a thanzi m'Malawi

Boma la Mangochi limapezeka chigwa cha mtsinje wa Shire. M'malo amenewa mumapezeza miyala ikulu ikulu yomwe ili ndi mlingo ochepa wa selenium ndi sulphur mu dothi lake. Zakudya zing'onozing'ono za mineral selenium ndi za sulphur amino acids ndizofunika ku kayandetsedwe ka chitetezo cha thupi la anthu a thanzi.

Cholinga cha phunziroli ndikuzindikira za selenium ndi sulphur amino acids mwa munthu amene ali wa thanzi. Phunziroli lidzaonetsanso zizindikiro za mtundu wa chakudya chomwe chili ndi selenium ndi suphur amino acids wambiri komanso ndimene chiwerengero cha anthu chimadyera m'bomali. Ndondomekoyi ikhonza kugwiritsidwa ntchito ngati mfundo pamene maphunziro owonjezera pa phunziroli adzachitike mtsogolo muno.

Zambiri za katengedwe ka chakudyachi zidzachitika kudzera mmafunso amene otenga nawo mbali adzayankhe atadya chakudya ndi kumwa tsikuli lisanafike. Akafukufuku adzayezanso sikelo ndi msinkhu wamunthu.

Oyenera kutengapo mbali ndani?
Anthu ena onse osankhika oyambira zaka 15 mpakana 60 amene ali athanzi. Onse otengapo mbali akhale kuti amakhala m'boma la Mangochi.

Uthenga kwa onse otenga mbali:
Dziwani kuti kutenga kwanu mbali mu phunziroli ndi kofuna nokha komanso opanda kukakamizidwa. Ngati mwagwirizana nazo kuti mutenga mbali mu phunzirili chonde siinani pa chi fomu chomwe alumikizacho. Dziwani kuti muli omasuka kusiya phunziroli nthawi ili yonse. Chiganizo chanu sichingakhale ndi vuto lili lonse kwa inu ngati munthu. Zitakhala choncho ndiye kuti zomwe mwapelekazo sizingagwiritsidwe ntchito.

Phunziroli lapangidwe ndi chithandizo chochokera ku Univesite ya Oslo (UIO). Akafukufuku alibe chithandizo chilichonse cha ndalama kuchokera kwa anthu osangalatsidwa ndi phunziroli kapena makampani. Phunziroli ndilovomelezedwa ndi a Ethical Committee ku University ya UIO, Norway ndi a College of Medicine Research and Ethics Committee ku Malawi.

Maganizo anu adzasungidwa mwa chinsinsi komanso ndi chitetezo.Palibe vuto lina lili lonse losowetsa mtendere kwa wina ali yense amene wadzipeleka kutenga nawo mbali pa phunziroli. A kafukuku alibenso udindo wina uli wonse opeleka chithandizo cha mankhwala kwa anthu otenga nawo mbali. Ngati mungafune kudziwa zambiri mukhonza kupeza Dr. Maleta ku College of Medicine pa Mangochi District Hospital Tel 08 232 202 kapena Stephano Mwaliwa, Tel 09692799..

Uthenga wa zotsatira
Pamapeto pa phunziroli (May 2007), Ofufuza adzatumiza chikalata cha momwe zotsatila zakhalira kwa ma District Health Officers oyenerela ndi mabungwe a zaumoyo amene ali

osangalatsidwa. Chikalatacho chidzakhalanso chopezeka kwa onse amene angatenge nawo mbali.

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Appendix 10: Invitation to controls - Chiyao

Akuwilangidwa kuti ajigale nawo mbali mu majiganyo ga:

Mtwe wa Pulojekiti: ”Katupe ka yakulya ya selenium ni sulphur amino acids mwa wandu wathanzi m’Malawi”.

Boma ja Mangochi jitemi mu chikhwawa chekulungwa. Mujele deraji mwana maganga gejinji gagakwete selenium ni sulphur jwa m’nono mu litaka lyakwe. Jele selenium ji ni sulphur amino acids jili jakusosekwa ku chiteteyo cha m’chilu ni umi wawandu.

Chakulinga cha gele majiganyoga ni chakuwungunya kajigale ka selenium ni sulphur amino acids mwa wandu wathanzi m’chilu. Gele majiganyoga tigapeleche litala lya kajigale ka yakulya ya gele magulu gawiriga. Gele majiganyoga tigalosye soni mtundu wa yakulya ukwete selenium ni sulphur amino acids jwa m’jinji nambo soni kuti akulidwa mwejinji uli ni wandu wa m’boma ja Mangochi. Yitupate pelepayi komboleka kamulichisya masengo mpala mfundo sya majiganyo gane gakusogolo patachiganisya majiganyo gane gakonjechesya. Utenga wakajigale ka yakulya tuchijigalidwa mkupitira kwausya mausyo wakujigala nawo mbali ni tachisala yosope yalire ni kumwa lisikulyo nkaniliyiche. Jwakaungunya tachalinga pasikelo soni kwalinga ulewu wawo.

Wani waliwakusosedwa kuti ajigale mbali?
Wandu wosope wali wathanzi wakwete yaka kutandira 15 mpaka 60. Wosope wakujigala nawo mbali awe wakutyochela m’boma ja Mangochi.

Utenga kwa wakujigala mbali
Amanyilire kuti kujigala kwawo mbali mu gele majiganyoga kuli kwakulipeleka, kwangachisya soni ngitwapa mbiya.
Naga ajitichisye kujigala nawo mbali pa gele majiganyoga, chonde asaine pachikalakala chalumbikenyocho. Atamose wawo akundile kujigala nawo mbali, amanyilire kuti wana ufulu wakugaleka majiganyoga ndawi jine jili jose. Nganisyo syawo pakutenda yeleyo pangali vuto kwa wawo mpela mundu. Naga ali atesile yaleyo nikuti utenga wosope waweleje alimkupeleka, ngasiukamulichisidwa masengo.

Majiganyoga gakukamuchisidwa ni wa University ja Oslo (UIO). Jwakaungunya jwangali chikamuchisyo cha mbiya kutyochela kwa gulu jawandu kapena makampani gakunonyelesedwa ni gale majigangyoga. Majigangyoga gali gakwitichika ni wa Ethical Committee ku UIO, Norway nambo soni wa College ja Medicine ni Ethics Committee ku Malawi.

Yitasalejeyo tiwe ya sili soni yakuteteyedwa. Pangali chakogoya chilichose kapena ngapata utendere kwa mundu jutalipeleche kujigala nawo mbali mu gele majigangyoga. Jwakaungunya jwangali udindo uliwose wakupeleka chikamuchisyo cha mtera kwa wandu wakujigala nawo mbali. Naga akusaka kumanyinlila jejinji wasimane Dr Maleta ku College ja Medicine ku chipatala chaboma ku Mangochi Tel 08 232 202 kapena Stephano Mwaliwa, Tel 09692799.

Utenga wa yakuyichisya

Patuchimalisya gele majiganyoga (May 2007), jwakaungunya tachitumisya m'ndandanda wa yakuyichisya kwa achakulungwakulungwa wa kulolela yipatala gagali gakunonyelesidwa. M'ndandandawo tuchiwa wakusimanikwa soni kwa wakujigala nawo mbali.

University of Oslo
Faculty of Medicine
International Health Department

Appendix 11: Concentform for controls - English

Declaration of consent for the study of

”Food intake of selenium and sulphur amino acids in healthy adults in Malawi.”

I am hereby declaring that I have received information on the study “Food intake of selenium and sulphur amino acids in healthy adults in Malawi.” I have been informed of the purpose of the above mentioned study, and that the information I will provide will be used for this study.

Besides, I have been informed that all the information I will provide will be treated with stringent confidentiality. Furthermore, I have been informed that the study has been approved by relevant authorities in Norway and Malawi.

I have also been notified that I can later withdraw from this study if I intend to do so in the future. I also know that the information pertaining to me can be used in similar study in the future.

Based on all this, I am hereby declaring the followings:

5. I agree voluntarily and without any reservation that I will participate in this study.

6. I agree that information I will provide can be used in similar study in the future.

Please cross on any item(s) to which you do not give your consent.

Participant signature Witness

Date and place Researcher signature, Frode Eick

Appendix 12: Concentform for controls – Chichewa

Declaration of consent for the study of

"Kadyedwe ka chakudya cha selenium ndi sulphur amino acids mwa anthu a thanzi m'Malawi".

Ndikunena pano kuti ndalandira ndondomeko ya phunziro "Kadyedwe ka chakudya cha selenium ndi sulphur amino acids mwa anthu a thanzi m'Malawi". Ndauzidwa za cholinga cha phunziro lomwe latchulidwa mmwambali, kuti zonse zomwe ndingapeleke zigwiritsidwa ntchito mu phunziroli.

Komanso ndauzidwanso kuti ndondomeko yonse yomwe ndingapeleke idzatetezedwa kwambiri. Ndauzidwanso kuti phunziroli ndilovomelezedwa ndi maudindo akuluakulu ofunikira a dziko la Norway ndi Malawi.

Ndauzidwanso kuti ndikhonza kusiya phunziroli ngati ndingafune mtsogolo muno. Ndikudziwanso kuti chilichonse chomwe ndingapeleke chidzagwiritsidwa ntchito mu phunziro ngati lomweli mtsogolo muno.

Pambali pa mfundo zonsezi ndikuvomeleza izi:

7. Ndikuvomeleza kuti mwakufuna kwanga ndimosakakamizidwa ndidzatengako mbali mu mphunziroli.

8. Ndikuvomeleza kuti zonse zomwe ndapeleka zikhonza kudzagwiritsidwa ntchito pa mphunziro lofanana ndi lino mtsogolo.

Chonde dulani malo onse omwe simupelekapo maganizo anu.

Saini ya Wotengambali Mboni

Tsiku ndi malo Saini ya wakafukufuku, Frode Eick

Appendix 13: Concentform for controls - Chiyao

Kusala yakwitika kutenda majiganyo ga:

” Katupe ka yakulya ya selenium ni sulphur amino acids mwa wandu wathanzi m’Malawi”.

Une apano ngusala kuti mbochele utenga wa majiganyo ga ”Katupe ka yakulya ya selenium ni sulphur amino acids mwa wandu wathanzi m’Malawi”. Asalire ya chakulinga cha majiganyo ga galembedwe penanipa nikuti utenga utimbeleche tuchikamulichisidwa masengo mu gele majiganyoga.

Kupatula pele, asalire kuti utenga utimbeleche tuchiwa wa asili soni wakuteteyedwa. Nambo soni asalire kuti majiganyoga gajitichisidwe ni achakulungwakulungwa wa maudindo gawo wa ku Norway ni wa ku Malawi.

Asalire soni kuti mpaka ngomboleche kuleka gele majiganyoga naga ndili sachile yeleyo ku sogolo kuno.

Payosopeyi, une ngusala ayi:

3. Ngwitika mwakulipeleka soni mwangasunga nganisyo sine kuti tinjigale nawo mbali pa gele majiganyoga.

4. Ngwitika kuti utenga utimbeleche komboleka kamulichisya masengo mu majiganyo gakulandana ni gelega kusogolo kuno.

Chonde afute malo galigose gankanagasaka.

Saini ja wakusaina: Mboni:

Lisiku ni malo: Saini ja wakafukufuku, Frode Eick

Appendix 14: Ethical approval – Norway

UNIVERSITY OF OSLO
FACULTY OF MEDICINE

To the relevant authorities

Institute of General Practice and Community Medicine
Section for International Health
P.O. Box 1130 Blindern
NO-0318 Oslo

Telephone: + 47 228 50 640
Telefax: + 47 228 50 607
E-mail:g.a.bjune@samfunnsmed.uio.no
URL: www.med.uio.no/ism/inthel

Date: June 23rd 2006
Your ref.:
Our ref.:

Ethical Review

Investigator's name: Eick, Frode

Title of the project: Food intake of selenium and sulphur amino acids in tuberculosis patients compared to healthy controls in Malawi.

Due to a re-organization in the Norwegian system for ethical review of research students' projects involving a second country, the project proposal has not been subject to a national review process this year.

The students have filled in the ordinary national form for ethical review of research projects involving human subjects and supplied the protocol for their project. A group of experts (medical research ethics, medical anthropology and clinical medicine) in our department have read the applications carefully and made their comments. The investigator's project is found to abide to international regulations, and the comments (below) are to guide the investigators to clarify, elaborate or modify some point(s) before they apply to their national authorities. In case there are such comments in this letter, the investigator's application will be corrected accordingly.

Comments of the reviewers:
I find the project both interesting and important as well. But I cannot find any description of method, and are you really going to interview 140 persons? It cannot be any kind of semi-structured interview in that case?
You also need to make an information and consent formula to the participants.

Yours sincerely,

Gunnar Bjune,
Professor International Health
Head of M.Phil. education in International Community Health

M.PHIL PROGRAMME
INTERNATIONAL COMMUNITY HEALTH
FACULTY OF MEDICINE
UNIVERSITY OF OSLO, NORWAY

Appendix 15: Ethical approval – Malawi

UNIVERSITY OF MALAWI

Principal

Prof. R.L. Broadhead, MBBS, FRCP, FRCPCH, DCH

Our Ref.: COMREC/16

Your Ref.: P.05/06/456

College of Medicine
Private Bag 360
Chichiri
Blantyre 3
Malawi
Telephone: 677 245
677 291
Fax: 674 700
Telex: 43744

21st August, 2006

Frode Eick
C/O Dr Maleta
Community Health Department
P/Bag 360
Blantyre 3

Dear Dr Maleta,

RE: P.05/06/456 – Food intake of senium and sulphur amoni acids in tuberculosis patients compared to healthy controls.

I write to inform you that COMREC reviewed your proposal which you resubmitted on 15th August, 2006. I am pleased to inform you that your proposal was reviewed and approved through an expedited process on 21st August, 2006. The approval was granted after considering that you addressed all the issues which were raised during the previous review.

As you proceed with the implementation of your study we would like you to take note that all requirements by the college are followed as indicated on the attached page.

Yours sincerely,

Prof J.M. Mfutso Bengo
SECRETARY - COMREC

Approved by
College of Medicine
2 1 AUG 2006
(COMREC)
Research and Ethics Committee

JMB/tck

Appendix 16: Letter from District Health Officer in Mangochi

Ref.NO.

MINISTRY OF HEALTH
MANGOCHI DISTRICT HOSPITAL
P.O. BOX 42
MANGOCHI.

DATE: 24th July, 2006

The Chairman,
College of Medicine Research and Ethics Committee
Private Bag 360
Chichiri
Blantyre 3

Att: Professor Joseph Mfutso Bengo

RE: P.05/06/457 – Investigation into plasma levels of selenium and glutathione in smear-positive adult tuberculosis patients and healthy controls in Malawi. by Heidi Arnsten

P.05/06/456 Food intake of selenium and sulphur amino acids in tuberculosis patients compared to healthy controls in Malawi by Frode Eick

The above named students have the permission of the management of Mangochi District Hospital to conduct their studies at our hospital subject to the studies being approved by your committee.

Yours sincerely,

George Chithope Mwale, MBBS, MPH
District Health Officer